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α-MSH Effects on Novelty-Induced Defecation, Plasma 11-OHCS And Whole Brain Catecholamines in Hypophysectomized Rats

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DATTA, P. C. AND M. G. KING. *α-MSH effects on novelty-induced defecation, plasma 11-OHCS and whole brain catecholamines in hypophysectomized rats.* PHARMAC. BIOCHEM. BEHAV. 12(5) 773–779, 1980.—The effect of hypophysectomy on novelty-induced defecation was found to be inhibitory in nature, but this effect was reversed by treatment with α-MSH. MSH-treated hypox rats maintained, over days, novelty-induced defecation and showed a significant elevation in plasma 11-OHCS levels, compared with the levels of control-injected counterparts. MSH treatment also reduced over Days 1 to 5 the whole brain DA and whole brain NE levels in hypox animals. Control-treated hypox rats, however, showed an increase in brain NE concentration which was significantly reduced by MSH treatment. Sham-hypox animals also sustained novelty-induced defecation after MSH treatment but did not show a significant rise in plasma 11-OHCS level. Whole brain DA and whole brain NE levels in sham-hypox rats were also reduced significantly by MSH treatment. The behavioral maintenance of defecation in hypox and sham-hypox rats seem to be concomitant with significant reductions in whole brain catecholamines.

α-MSH Novelty Hypophysectomy Defecation Plasma 11-OHCS Whole brain DA Whole brain NE

IT WAS demonstrated by Datta and King [4] that animals treated with α-melanocyte-stimulating hormone (α-MSH) sustain a learned Passive Avoidance Response (PAR) and also sustain Passive Avoidance (PA)-induced defecation for a longer period than animals treated with a control solution. Hypophysectomized (hypox) rats, compared with sham-hypox or intact rats, show poorer acquisition and retention of the PAR and also exhibit less PA-induced defecation [6]. When treated with α-MSH, hypox rats perform as well as intact or sham-hypox rats injected with α-MSH, and show markedly better retention of the PAR than either intact or sham-control rats injected with a diluent solution [6]. These observations have substantiated the finding that the effects of MSH observed on the learning and retention of a PAR and on PA-induced defecation [4] are induced by MSH probably in conjunction with brain catecholamines (CAs) independently of other pituitary hormones [5].

Datta and King [5] have demonstrated further that MSH can sustain novelty-induced defecation and that this maintenance of defecation responses is accompanied by a concomitant significant reduction of whole brain norepinephrine (NE) and whole brain dopamine (DA) concentrations over days of treatment. These findings should be verified using hypox animals to determine whether the reductions of brain NE and DA concentrations are mediated by MSH alone, independently of other pituitary hormones, or in conjunction with other pituitary hormones. The involvement of pituitary secretions in stress-responses is well established [2,25]. Versteeg *et al.* [34] observed a decrease in the turnover of whole brain 5-hydroxytryptamine (5-HT), NE and DA after hypophysectomy, but they did not observe any difference between the hypox and the sham-operated animals in brain NE and DA turnover when those animals were treated with $MSH/ACTH_{4-10}$. On the other hand, Kostrzewa *et al.* [19] reported that for rats treated with MSH a lowering of brain NE occurred only in hypox rats. When treated with MSH the hypox rats also showed a decrease in brain DA disappearance, i.e., increased available brain DA, in hypox rats during the first six hours. Thus it may be expected that the effects of MSH observed by Datta and King [5] on brain NE and DA concentrations in intact rats would be different from the effects in hypox rats [11, 19, 31]. It is therefore important to examine the effects of MSH on the changes in concentrations of whole brain NE and DA in hypox rats, relative to those changes in sham-hypox rats, and also to show whether MSH-treated hypox rats sustain novelty-induced defecation in a similar way to MSH-treated sham-controls.

On the basis of the observations of Datta and King [5] and those made by Kostrzewa *et al.* [19], it is predicted that MSH treatment, relative to the diluent injection, will sustain

longer novelty-induced defecation and also concomitantly reduce whole brain NE and whole brain DA concentrations over days of treatment.

An important observation made by Datta and King [5] was that α-MSH treatment lead to a significant rise of plasma 11-hydroxycorticosterone (11-OHCS) levels in intact rats in the resting, home cage conditions, whereas MSH treatment, relative to control injection, failed to produce a significant steroidogenic effect in rats exposed to a novel environment on several days. A probable explanation proposed for these different steroidogenic effects of MSH in resting and behavioral conditions was that MSH might have increased the awareness of the animal and thus modulated the 11-OHCS release acting centrally; alternatively the effects of MSH might have interacted with the effects of endogenous ACTH release to novelty exposure in an inhibitory fashion [2,17]. An attempt to answer this question calls for a demonstration of the effects of MSH on 11-OHCS release independently of other pituitary hormones using hypox and sham-hypox rats. Accordingly, the main aim of this experiment was to demonstrate the independent effects of α-MSH on novelty-induced defecation and concomitant changes in plasma 11-OHCS levels, brain NE and brain DA concentrations on successive days of MSH treatment combined with novelty exposure.

In this experiment only Day 1 and Day 5 samples were tested for the effects of MSH in hypox rats, because it has been reported [5] that there are significant effects of MSH on defecation, brain NE and brain DA concentrations on Day 5 relative to those on Day 1.

METHOD

Animals

The animals used in this experiment were 24 hypox and 24 sham-hypox naive male Wistar rats, 90–100 days old at the start of novelty testing. Following the practice of Datta and King [6] animals were operated at 60–70 days and after surgery were allowed 4 weeks recovery period during which time they were adapted to a 12/12 hr light/dark cycle in the animal holding room. Two animals were kept in each cage. Temperature in the room was maintained at 23±1°C.

Hypox and sham-hypox animals were randomly assigned to one of the following treatment duration groups: 12 hypox and 12 sham-hypox rats for the Day 1 group, and 12 hypox and 12 sham-hypox rats for the Day 5 group. In each treatment duration group rats were again randomly assigned (six animals per group) to either α-MSH treatment or its control treatment.

Apparatus

The novel environment was provided by a 35×35×45 cm Plexiglas box, described elsewhere by Datta and King [4], with a grid floor consisting of 0.5 cm diameter brass rods set at 1 cm apart. There was a 15×15 cm Plexiglas platform in one corner of the box which was fixed at 3.5 cm above the grid floor. The platform was covered with masking tape to stop the animals from slipping and the box was externally covered with opaque paper except the top which was left open. A metal tray covered with paper towelling was located 9 cm below the grid floor. The platform was attached to a micro-switch which was wired to a mini-computer. The computer operated an electronic clock and a print-out timer. The test box was housed in a sound-lagged, air-conditioned (23±1°C) cubicle with the ambient level of illumination in the box and the cubicle at 35 lx.

Other equipment included a stereotaxic apparatus (described by Everitt and Cavanagh [10] and by Koyama [20]).

Procedure

Animals were adapted to a 12/12 hr light/dark cycle for 4 weeks and handled for 3 min each day for 7 days following the procedure of Datta and King [5].

Surgical Procedure

The surgical procedure employed in this experiment was the intra-aural technique described by Datta and King [6]. Animals were operated on under mild ether anesthesia which was maintained by placing a Penthrane soaked pad on the nostrils of the rat during surgery. After fixing the rat in the stereotaxic apparatus, the probe needle was slowly inserted in the right auditory canal using a rotating motion and then slowly removed. A 5 ml syringe with a 51×1.25 mm needle loaded with 2 ml of a 0.9% NaCl solution was then slowly inserted into the hollow of the right ear bar with the bevel facing up. With 4 mm to travel the needle was rotated 180° counterclock-wise to avoid rupture of the sinus and was then slowly pushed the remaining distance. The pituitary capsule was broken with a rotating movement of the needle and with the bevel facing down the pituitary was gently drawn out. If the amount of extirpated pituitary material visible in the syringe was assessed as adequate (about 1 mg), the needle was withdrawn from the ear bar, the rat was removed from the stereotaxic apparatus and injected with 2.5 mg cortisone acetate (Cortisyl) and 1 ml of a solution containing 1% oxytetracycline hydrochloride and 5% glucose. The operated animal was then wrapped in gauze for about 1 hr and transferred to its home cage where it was maintained on moist chow and 5% sucrose in water. The same operation procedure was followed for sham-hypophysectomy with the exception of breaking of the pituitary capsule and removal of the gland. Sham-hypox rats did not receive the cortisone acetate injection.

In all, 45 rats were hypophysectomized of which 6 died subsequently and another 15 rats were discarded due to incomplete hypophysectomy. A group of 26 animals were sham-hypophysectomized of which 2 rats were discarded for weight loss assessed on the weekly weight chart.

Injection Procedure

Each day each hypox or each sham-hypox rat received an IP injection of either synthetic α-MSH (supplied generously by CIBA-GEIGY) (10 μg/rat) or the diluent solution (0.9% NaCl+0.01 M acetic acid) following the practice of Datta and King [4]. The hypox and the sham-hypox animals received injections in accordance with the following schedule: animals in the Day 1 group were injected for 1 day only, and animals in the Day 5 group were injected each day for 5 days.

Behavioral Procedure

Following the practice of Datta and King [5] each animal was placed on the platform in the test box 20 min after injection. As soon as the rat was placed on the platform, the electronic timer started and when the rat stepped down the timer printed out the step-down latency. Only the initial latency was recorded and the electronic clock timed out the 60 sec duration in the test box. On the completion of 60 sec the

rat was transferred to its home cage. Fecal boluses were collected from the box and tray and counted. After each rat the box was cleaned and dried before the next animal was brought in.

Both the hypox and the sham-hypox animals in the Day 1 duration group were exposed to the test box for 1 day only, and those animals in the Day 5 duration group were exposed to the test box at the same time each day for 5 days.

Physiological and Biochemical Procedures

The hypox and the sham-hypox rats in each treatment duration group were sacrificed 21 min after injection on the final day scheduled for that duration group. As soon as the rat completed 60 sec in the test box it was taken out and immediately decapitated. The trunk blood was collected in heparinized tubes and centrifuged at 4000 rpm for 15 min. The plasma was immediately collected and frozen in acid-washed premarked vials. As soon as the blood was collected, the brain was removed from the skull, weighed and homogenized in 5 ml of a solution of 1% sodium metabisulphate and 3.7% perchloric acid [5]. While being homogenized with plastic trephines the samples were kept in dry ice, and after the homogenizing was complete the samples were centrifuged for 10 min at 4000 rpm and supernatants were collected. The samples were then homogenized and centrifuged for 10 min for the second time and the supernatants were again collected and frozen.

The assay for plasma 11-OHCS levels was done by the fluorimetric method described by Mattingly [22]. Two estimations for each sample were taken. The assays of the samples for brain DA and brain NE were carried out on the same day following the procedure described by Haggendal [12] and Hinterberger [13]. Both plasma 11-OHCS and brain CAs were assayed using a fluorescence spectrofluorimeter (Perkin-Elmer, Model MPF-4).

RESULTS

The behavioral measures analyzed were the transformed scores ($\log_{10}$ of X) of the step-down latencies and the transformed scores ($X = \sqrt{X+0.5}$) of the number of boluses on Days 1 and 5 [35]. The biochemical measures analyzed were the plasma 11-OHCS levels (μg/100 ml) and whole brain DA and whole brain NE levels (ng/g of wet tissue). The data for the hypox and the sham-hypox animals were first analyzed separately and then they were analyzed together.

The mean values for the bolus frequencies, plasma 11-OHCS levels, brain DA and brain NE levels of the MSH treated and the control-injected hypox rats are shown in Fig. 1. Step-down latencies are not included in Fig. 1 because the difference between the drug-treated and control-injected groups was not significant. However, the mean values with standard errors for the step-down latencies (sec) of MSH-treated and control-injected hypox animals were 7.37 ± 2.16 and 6.41 ± 1.67 respectively in the Day 1 duration group, and 9.81 ± 3.50 and 6.95 ± 0.94 respectively in the Day 5 duration group. Results of 2-way ANOVA with repeated measures showed that α-MSH treatment, relative to its control treatment, significantly enhanced and sustained high novelty-induced defecation on Days 1 and 5, $F(1,10)=23.79$, $p<0.001$. The interaction effect of MSH treatment with days of exposure to novelty was also significant, $F(1,10)=13.55$, $p<0.01$, on the defecation measure. Further analysis of the days effect of the individual drug treatment groups revealed that hypox rats treated with the control solution showed a

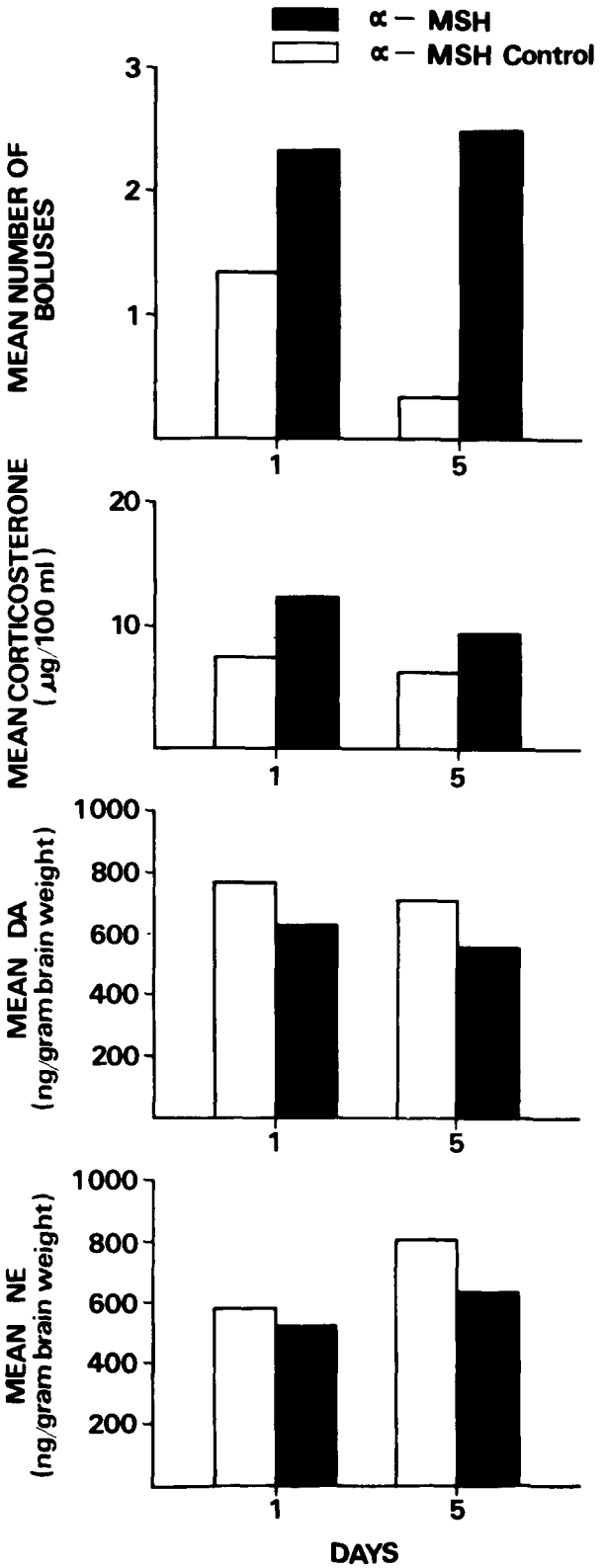

FIG. 1. Mean values for the number of boluses, plasma 11-OHCS, whole brain DA and whole brain NE levels of α-MSH-treated and control-treated hypox animals over Days 1 and 5 of novelty exposure.

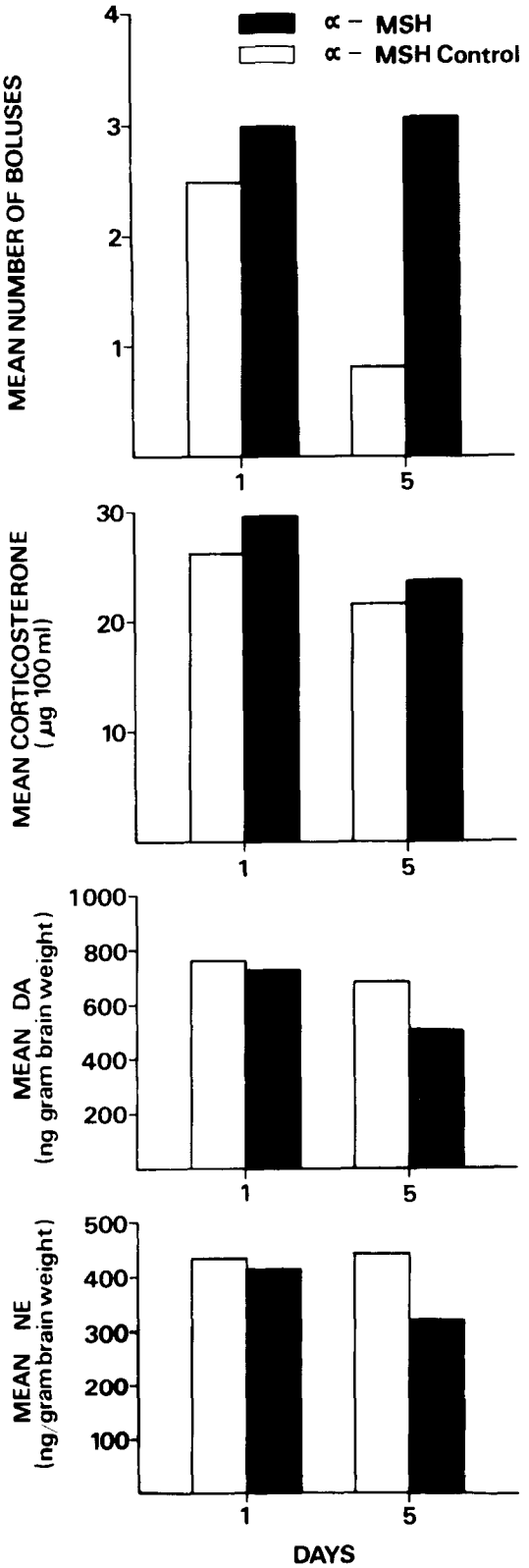

FIG. 2. Mean values for bolus frequencies, plasma 11-OHCS levels, whole brain DA and whole brain NE levels of MSH-treated and control-injected sham-hypox animals over Days 1 and 5.

significant diminution of novelty-induced defecation responses over days of exposure to the same environment, $F(1,5)=14.04$, $p<0.01$.

Analysis of plasma 11-OHCS levels in hypox animals showed that MSH treatment, relative to its control injection, also increased the plasma 11-OHCS levels, $F(1,10)=6.45$, $p<0.05$. These findings were consistent with the increased plasma 11-OHCS levels observed in MSH-treated hypox rats exposed to a PA situation [6].

Results of a 2-way ANOVA with repeated measures for whole brain DA levels showed that MSH treatment significantly reduced brain DA in hypox rats compared with the DA in control-injected hypox rats, $F(1,10)=8.29$, $p<0.05$. Reduction in brain DA was apparent on the first day (Day 1) of the combined MSH and novelty treatment, whereas novelty exposure alone did not change the brain DA of control-treated hypox animals (see Fig. 1).

A 2-way ANOVA showed that the concentrations of the whole brain NE in the hypox rats were significantly reduced by daily MSH treatment when compared with concentrations after the control treatment, $F(1,10)=9.85$, $p<0.01$. The overall days effect was also significant, $F(1,10)=23.15$, $p<0.001$, indicating a significant increase in the concentrations of NE in hypox animals over days of exposure to the novel environment (see Fig. 1). However, separate analysis of the days effect for the individual drug groups showed that novelty exposure itself increased the brain NE levels of the control-injected hypox rats, $F(1,5)=32.30$, $p<0.001$, whereas the brain NE level was not significantly increased for the MSH-treated hypox animals even though there was a slight elevation of brain NE on Day 5 (see Fig. 1).

Sham-hypox Animals

In Fig. 2 are shown the mean values of bolus frequencies, plasma 11-OHCS, brain DA and brain NE levels in sham-hypox animals on Days 1 and 5. ANOVA revealed that step-down latency of sham-hypox animals was not affected by either MSH or its control treatment. The mean step-down latencies (sec) were 9.41 ± 6.99 for MSH-treated rats and 9.60 ± 1.65 for control-treated rats on Day 1, and on Day 5 were 8.75 ± 2.40 for MSH-treated rats and 8.03 ± 2.62 for control-injected rats.

A 2-way ANOVA with repeated measures showed that MSH treatment significantly sustained novelty-induced defecation in sham-hypox animals, $F(1,10)=14.91$, $p<0.01$. The overall days effect, $F(1,10)=4.70$, $p<0.05$, and the interaction effect, $F(1,10)=6.37$, $p<0.05$, were also found to be significant. In an attempt to explain the interaction effect, days effects were tested for the individual drug treatment groups and it was found that the defecation of control-treated sham-hypox rats habituated significantly, $F(1,5)=6.59$, $p<0.05$, whereas the defecation of their MSH-treated counterparts did not habituate over days.

Analysis of plasma 11-OHCS levels using a 2-way ANOVA showed that α-MSH treatment did not have a significant effect on the plasma 11-OHCS levels in sham-hypox animals exposed to a novel environment over Days 1 and 5. This finding was consistent with the observation of a non-significant effect of α-MSH on plasma 11-OHCS in intact rats exposed to novelty [5]. However, there was a slight elevation of plasma 11-OHCS in both MSH-treated intact rats [5] and MSH-treated sham-hypox rats (see Fig. 2).

Analysis of brain DA in sham-hypox animals using a 2-way ANOVA showed that α-MSH treatment significantly reduced brain DA in sham-hypox animals over Days 1 and 5,

$F(1,10)=5.63$, $p<0.05$. The overall days effect, $F(1,10)=18.54$, $p<0.01$, was also significant which suggests a systematic reduction in brain DA (because of the absence of interaction effect) in both MSH- and control-treated sham-hypox animals, an observation similar to that for intact animals exposed over days to novelty. However, results from multiple Scheffé comparisons [35] carried out *post hoc* showed that for MSH-treated sham-hypox animals there was a significant ($p<0.05$) reduction in brain DA on Day 5 (Mean=538.41 ng) compared with the DA on Day 1 (Mean=750.93 ng) whereas the reduction of DA was not statistically significant for the control-treated sham-hypox animals. Consistent with the observation made by Datta and King [5] the results of a 2-way ANOVA with repeated measures for brain NE showed that α-MSH treatment, relative to control treatment, significantly decreased brain NE in sham-hypox rats, $F(1,10)=17.59$, $p<0.01$. The overall days effect, $F(1,10)=7.07$, $p<0.05$ and the interaction effect, $F(1,10)=10.16$, $p<0.01$, were also significant. Subsequent analysis with ANOVAs showed that α-MSH treatment significantly reduced brain NE in sham-hypox animals exposed over days to novelty, $F(1,5)=26.06$, $p<0.01$, whereas the brain NE levels of control-treated sham-hypox animals remained unchanged over days of novelty exposure. These findings were also similar to the findings with intact animals treated with MSH and novelty [5].

Hypox vs Sham-Hypox Animals

A 3-way ANOVA showed that step-down latencies were not affected by hypophysectomy, drug treatment or days of exposure to a novel environment. Results of the 3-way ANOVA with repeated measures for bolus frequencies of hypox and sham-hypox animals showed that novelty-induced defecation was significantly reduced by hypophysectomy, $F(1,20)=4.91$, $p<0.05$. However, when treated with MSH, hypox and sham-hypox animals did not differ, and there was a slight difference between control-treated hypox and sham-hypox animals. The drug treatment effect due to MSH was highly significant, $F(1,20)=38.45$, $p<0.001$, which showed the effectiveness of α-MSH in sustaining novelty-induced defecation in both hypox and sham-hypox animals. The significant overall days effect, $F(1,20)=6.48$, $p<0.05$, and the significant interaction effect of days and drug treatment, $F(1,20)=16.93$, $p<0.001$, were due mainly to the habituation of defecation in control-treated hypox and sham-hypox animals over days, $F(1,10)=15.38$, $p<0.001$.

Analysis of plasma 11-OHCS levels for hypox and sham-hypox animals revealed that plasma 11-OHCS levels were significantly affected (reduced) by hypophysectomy, $F(1,20)=89.48$, $p<0.001$, and drug treatment did not make an appreciable difference to the plasma levels of 11-OHCS in hypox and sham-hypox animals exposed to novelty. The overall days effect was significant, $F(1,20)=4.26$, $p<0.05$, showing an habituation effect on 11-OHCS over days of exposure to novelty.

Results of a 3-way ANOVA with repeated measures for the brain DA levels of hypox and sham-hypox animals showed that hypophysectomy per se did not change brain DA significantly, but treatment with α-MSH was found to reduce brain DA significantly over days of exposure to the test box, $F(1,20)=13.73$, $p<0.001$. The overall days effect was also highly significant, $F(1,20)=14.34$, $p<0.001$. In subsequent analysis with a 2-way ANOVA it was revealed that brain DA was reduced in hypox and sham-hypox animals (combined days effect) but only by MSH treatment, $F(1,10)=16.57$, $p<0.001$. The interaction effect of hypophysectomy and days exposure on the DA scores of MSH-treated animals was significant, $F(1,10)=5.65$, $p<0.05$, apparently due to the fact that in sham-hypox animals (from 750.93 ng to 538.4 ng, significant at $p<0.05$ on the Scheffé test) the reduction in brain DA was greater than the reduction of brain DA in hypox counterparts (from 617.25 ng to 561.45 ng, not significant on the Scheffé test) after MSH treatment.

The results on brain NE of hypox and sham-hypox animals obtained from a 3-way ANOVA with repeated measures showed that hypophysectomy significantly elevated brain NE levels in animals exposed to novelty, $F(1,20)=207.79$, $p<0.001$. Treatment with α-MSH was found to reduce the levels of brain NE in hypox and sham-hypox animals, $F(1,20)=21.99$, $p<0.001$, over days. The overall days effect, $F(1,20)=12.69$, $p<0.01$, interaction effect of days and hypophysectomy, $F(1,20)=30.83$, $p<0.001$, and the interaction effect of days with drug treatment, $F(1,20)=6.40$, $p<0.01$, were all significant, which invited subsequent analysis for individual drug groups. Subsequent analysis with a 2-way ANOVA for brain NE levels of α-MSH-treated hypox and sham-hypox animals showed that the brain NE level in hypox rats was significantly higher than the NE level in sham-hypox rats which showed a significant reduction over days, $F(1,10)=62.21$, $p<0.001$. The interaction effect of hypophysectomy and days of exposure was also significant, $F(1,10)=11.45$, $p<0.01$, which indicated an uneven reduction for brain NE in hypox and sham-hypox animals. As can be seen in Fig. 1 the brain NE level of MSH-treated hypox animals relative to control-treated counterparts, even though reduced by MSH treatment, actually increased slightly over days. Comparison between control-treated hypox and sham-hypox animals with a 2-way ANOVA, showed that the hypophysectomy effect, $F(1,10)=219.40$, $p<0.001$, days effect, $F(1,10)=25.68$, $p<0.001$, and the days×hypox interaction effect, $F(1,10)=22.45$, $p<0.001$, were significant. In control-treated hypox rats, there was a significant increase in brain NE over days, whereas in control-treated sham-hypox animals there was no appreciable change in brain NE over days. The above results of ANOVAs demonstrated the significant effect of hypophysectomy (of the pituitary hormones in general and of MSH in particular) and of α-MSH treatment on novelty-induced defecation, plasma 11-OHCS, whole brain DA and NE concentrations over days of exposure. These results also explicate the effect of novelty stress on the whole brain level of NE in hypox animals.

DISCUSSION

The results of this experiment support the explanation that α-MSH increases and sustains novelty-induced defecation by its own action, independently of other pituitary hormones. MSH treatment not only exacerbated the defecation responses of hypox rats but also maintained this response for at least 5 days. This effect of MSH on the maintenance of novelty-induced defecation responses of hypox rats was similar to the maintenance effect obsrerved of MSH-treated sham-hypox or of intact rats [5]. The observation of increased and sustained novelty-induced defecation of MSH-treated hypox rats supported and replicated the observed enhancement and sustained PA-induced defecation responses of MSH-treated hypox rats [6].

The present results also demonstrate that MSH is capable

of increasing significantly plasma 11-OHCS levels independently of pituitary ACTH. This observation confirms the observed steroidogenic effect of MSH in rats in the home cage resting condition [5] and also the steroidogenic effect of MSH in hypox animals following extinction of a PA task [6]. As is indicated in Fig. 1, MSH treatment elevated 11-OHCS levels in hypox rats on Day 1, but there was a slight habituation of this level on Day 5. The same slight habituation effect (non-significant) on the plasma 11-OHCS levels in sham-hypox rats was observed on Day 5 compared with Day 1 (see Fig. 1). However, the increased 11-OHCS levels in hypox rats following MSH treatment support the contention of Lebovitz *et al.* [21] and Kakhan *et al.* [14] to the effect that α-MSH has significant in vivo and in vitro steroidogenic effects even though it is only about 1% of the steroidogenic effect of ACTH. Because animals were decapitated immediately after novelty exposure and because one rest day before the next exposure was sufficient time for the 11-OHCS level to return to the basal level [25], novelty exposure did not show a significant effect on plasma 11-OHCS on Days 1 and 5. But as has been mentioned by Datta and King [5] increased awareness following upon MSH treatment may modulate a central function or may set-up a central autonomic tone [24] which can quickly regulate the 11-OHCS secretion through neural transmission. The central autonomic tone, if modulated and maintained by MSH, may well serve to explain the maintenance of defecation (PA-induced or novelty-induced) and the increased and subsequently habituated plasma 11-OHCS levels needed for the metabolic reactions. Such a sustained autonomic tone may also explain the occurrence of many other behaviors observed by previous investigators which could be regarded as arousal responses [8, 17, 24, 27, 29, 32].

The reductions of whole brain DA and whole brain NE after MSH treatment and novelty exposure in hypox as well as sham-hypox animals demonstrates that such reductions of brain CAs were probably mediated by MSH independently of other pituitary hormones. The reduction of DA commenced on Day 1 and was sustained on Day 5 after MSH treatment (see Figs. 1 and 2). After the reduction of DA on Day 1, MSH-treated hypox rats did not show a further significant reduction on Day 5 relative to the level on Day 1, but in MSH-treated sham-hypox rats there was a further reduction of brain DA on Day 5 relative to the level on Day 1. This reduction of brain DA on subsequent days of exposure to novelty observed in sham-hypox rats has also been observed in intact rats [5]. The greater reduction of brain DA in MSH-treated hypox rats, relative to MSH-treated sham-hypox rats, on Day 1 could be due to the fact that exogenous MSH does not become localized (uptaken) in the pituitary [9,18] to inhibit its own secretion [18,27] and therefore could act directly on the CA concentrated areas in the brain and on the pineal [18]. Besides, there is the possibility of increased sensitivity of the CNS to α-MSH due to the lack of availability of circulating MSH for more than 4 weeks. The reason why the pineal gland is thought to be involved in the sharp reduction of DA on Day 1 in MSH-treated animals is that MSH has been reported to affect Melatonin secretion from the pineal and that in the absence of MSH (and other pituitary hormones) there is a significant increase in plasma Melatonin levels [15, 16, 18, 33]. But since only one MSH dose was administered per day, the pineal might have increased the release of Melatonin which might have subsequently abated the reduction of brain DA on Day 5 relative to Day 1 in MSH-treated hypox animals (see Fig. 1). Of relevance is the observation that Melatonin treatment over 5 days significantly increases the brain DA levels in rats exposed to novelty [7].

The implication of pineal Melatonin or hypothalamic MIF-I seems to be more conspicuous in the whole brain NE levels of MSH-treated and control-treated hypox rats. The NE levels of the hypox rats were higher on Day 5 relative to the level on Day 1, even though MSH treatment, relative to control injection, significantly reduced the NE level over days of exposure to novelty. The effect of novelty stress on Melatonin release from the pineal and the possibility of Melatonin acting as an inhibitory neuroregulator have been noted previously [1, 4, 7, 23]. Melatonin has also been reported to affect the release of hypothalamic MIF-I [7,33] which has been recently reported to increase the levels of brain DA and NE in animals exposed to novelty and to act on the central CA pathways [7,17]. Hence hypox rats exposed to a novel environment may respond to stress by an increased release of Melatonin from the pineal in the absence of the pituitary which previously has been reported to release both MSH and ACTH under both physical and psychological stresses [28]. The increased release of Melatonin in hypox rats, by acting at the synapses, may reduce the release and disappearance of brain DA and NE which are regarded as inhibitory neurotransmitters [3, 7, 23].

The present findings on NE levels in MSH-treated hypox animals support the findings of Kostrzewa *et al.* [19] to the effect that there was a lower rate of NE disappearance (increased brain NE) in hypox rats during the first four hours after MSH treatment. It can be said therefore that even though behavior was altered by MSH in both hypox and sham-hypox animals in the same direction, the neurotransmitter changes, especially NE changes, were not exactly in the same direction. These findings support the observations and contentions of Kostrzewa *et al.* [19] and of Kastin *et al.* [17] concerning the reduction of brain CAs in hypox animals after MSH treatment and novelty exposure, but do not support the observations made by Versteeg *et al.* [34] to the effect that administration of MSH/ACTH to hypox rats does not change the disappearance of brain NE.

However, the changes in the concentrations of brain DA and NE in MSH-treated hypox animals support the earlier contention [5] that brain CAs might be involved in the maintenance of novelty-induced defecation responses and that these changes in brain CAs, especially NE, might be mediated by MSH independently of other pituitary hormones. The theory of Schildkraut and Kety [30] and of Bernard *et al.* [3] that brain CAs are implicated in emotional responses in rats seems to be partially supported by the observed reduction of brain NE in sham-hypox rats. When treated with MSH, hypox rats also maintained novelty-induced defecation and showed concomitant significant reductions in brain NE level relative to the NE level in the control-treated hypox counterparts over days of novelty exposure.

The results of step-down latencies (activity) in this experiment have reconfirmed the observations of Datta and King [4,5], Kastin *et al.* [16] and Roeder [26] that MSH does not alter significantly the initial activity (step-down latency) of rats exposed to a novel environment. These findings on step-down activity also show that the delayed extinction of a PAR after MSH treatment in hypox rats [6] was not due to any immobilizing effect of MSH on locomotor behavior.

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