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α-MSH and MIF-I Effects on Catecholamine Levels and Synthesis in Various Rat Brain Areas[1]

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KOSTRZEWA, R. M., A. J. KASTIN AND M. A. SPIRTES. *α-MSH and MIF-I effects on catecholamine levels and synthesis in various rat brain areas.* PHARMAC. BIOCHEM. BEHAV. 3(6) 1017–1023, 1975. – Attempts were made to find a biochemical correlate with previously observed behavioral alterations after administration of α-melanocyte-stimulating hormone (MSH) and MSH release-inhibiting factor (MIF-I). Brains of intact and hypophysectomized (hypox) rats were analyzed for endogenous catecholamine levels and the disappearance rate of endogenous norepinephrine (NE) after treatment with the tyrosine hydroxylase inhibitor α-methyl-para-tyrosine (AMPT). The studies undertaken show the following: (1) After the injection of MSH (100 μg/kg IP daily × 3) and AMPT, samples in different groups of intact and hypox rats were taken at 0, 1, 2, 4 and 6 hrs in 7 different brain areas. In the mid-brain area for the intact group of rats, the rate of disappearance of NE was faster and for the hypox rats it was slower than the rate for control rats not treated with the peptides. NE levels in the same area at time 0 were 11 percent lower than controls in hypox rats and unchanged in unoperated animals. (2) After the injection of MIF-I (20 mg/kg IP daily × 3) in similar experiments as with MSH, a reduced rate ($p<0.05$) of NE disappearance for the first 4 hr and an increased rate ($p<0.05$) of NE disappearance for the last 2 hr of the experiments occurred for both the intact and hypox rats in the mid-brain area where endogenous NE levels were lowered by 11 and 12 percent at 0 min. In no other brain areas were alterations in NE breakdown found in both the intact and hypox rat groups. Behavioral changes have been found previously under similar experimental conditions in both intact and hypox rats. (3) Rates of dopamine disappearance in experiments similar to those described for NE disappearance indicated that in the striatal brain area no change was found in the intact rats after either MSH or MIF-I, whereas a decrease in DA disappearance was found for hypox rats during the six hour experimental period only after MSH. The results indicate that a correlation between behavioral changes, rates of disappearance and endogenous levels of NE in the mid-brain area may occur after MIF-I at the times examined but that a similar correlation for MSH did not appear likely.

Melanocyte-stimulating hormone Melanocyte-stimulating hormone release-inhibiting factor Hypophysectomy Catecholamines

SEVERAL studies in the past few years have attempted to associate melanocyte-stimulating hormone (MSH) or an MSH-release inhibiting factor (MIF-I; MRIH; prolyl-leucyl-glycinamide) with assorted behavioral alterations in humans and animals [17]. MIF-I has been shown to potentiate the motor behavioral effects of dihydroxy-phenylalanine (DOPA) in rats [21], and preliminary clinical studies with human subjects have shown that MIF-I alone has some antiparkinsonian activity [3, 8, 13]. In addition, Friedman *et al.* [9] stated that MIF-I decreased endogenous levels of dopamine (DA) in rat striatum and increased the *in vitro* rate of synthesis of ^{3}H-DA from ^{3}H-tyrosine by slices of striatum. However, Plotnikoff *et al.* [25] reported that the levels of DA and homovanillic acid (HVA) were unaltered in the caudate nucleus of the brain after MIF-I. It was also observed that MIF-I antagonized the tremors induced by oxotremorine in mice [22,23] and reversed the sedative effects of deserpidine in mice and monkeys [24]. It further has been reported that MIF-I does not alter the metabolism of exogenously administered DOPA in rats [2], although critical analysis of the data suggests that O-methylation of DOPA may be impaired [26].

Effects on the EEG [15,19] and somatosensory-evoked responses in man [16] and the rat [27] have been described for MSH and its active component $ACTH_{4-10}$ (=MSH). Reports are also available indicating that MSH affects conditioned avoidance responding [5,6], appetitive responses [14, 28, 32], and passive avoidance responses [4,29]. Studies by Leonard [18] with the peptide fragment $ACTH_{4-10}$ which is intrinsic to the structure of the naturally occurring pituitary hormone MSH, indicated that $ACTH_{4-10}$ may have slightly increased DA levels in whole

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rat brain and hindbrain (i.e. tegmentum, colliculus, pons and medulla), while DA turnover reportedly was increased in the midbrain (i.e. hippocampus, striatum, thalamus, hypothalamus and amygdaloid cortex). Also, endogenous levels of NE were found to be elevated in rat midbrain [18] in the same experiments, and the turnover of NE was reportedly increased in whole rat brain and other brain regions [18].

The present study was undertaken to further investigate whether, under our conditions, the behavioral effects of MIF-I or MSH were accompanied by alterations in catecholamine levels or catabolism in discrete regions of the brain. Our results indicate that, although the midbrain of both unoperated and hypophysectomized rats is the only area for which both behavioral and NE catabolism changes can be ascertained after treatment with either MSH or MIF-I, a correlation has not been definitely found as yet between the behavioral and biochemical alterations after treatment of the rats with the above-named hormones. A number of other selective effects of these hormones on other brain areas was also found. These results do not wholly agree with the findings of other investigators.

METHOD

Animals and Procedure

Effect of MIF-I and MSH on endogenous levels of catecholamines in various regions of rat brain. Male, albino rats, 160–185 g, received a total of 3 injections of MIF-I (20 mg/kg IP) or MSH (100 μg/kg. IP) at 24 hr intervals, and were sacrificed by decapitation 24 hr after the final dose. At the time of sacrifice, brains were rapidly removed and dissected according to Glowinski and Iversen [10]. Tissues were frozen on dry ice and stored at −50°C until assayed for catecholamines.

Effect of MIF-I and MSH on turnover of catecholamines in various regions of rat brain. Rats, 140–170 g, received a total of 3 injections of MIF-I (20 mg/kg IP) or MSH (100 μg/kg IP) at 24 hr intervals. Fifteen min after final treatment with MSH or one hr after final treatment with MIF-I, rats received the tyrosine hydroxylase inhibitor α-methyl-p-tyrosine methyl ester hydrochloride (AMPT) (300 mg/kg IP, free base) obtained from Regis Chemical Co., Morton Grove, Ill. Animals were sacrificed at intervals of 1, 2, 4, and 6 hr after injection of AMPT and brain regions were removed for catecholamine analysis. Zero time controls were sacrificed at the time when MPT would otherwise have been administered. Since control NE and DA values in various parts of the brain were always the same 15 or 60 min after the last hormonal treatment, all saline controls were sacrificed or treated with AMPT, 15 min after saline injection.

Effect of MIF-I and MSH on apomorphine-induced stereotypy in rats. In this experiment, rats received varying amounts of apomorphine (Merck) (1–5 mg/kg IP) 1 hr after pretreatment with MIF-I (20 mg/kg IP) or 15 minutes after MSH (100 μg/kg IP). As controls, appropriate groups of rats received only MIF-I or MSH with the second treatment consisting only of the vehicle for apomorphine, or the vehicle for the hormone followed by apomorphine. Activity was observed over a period of 1 hr after the injection of apomorphine or vehicle, and the stereotypy of the rats was graded according to Ernst [7]. A dose of about 2.75 mg/kg IP was chosen which allowed the rats employed to reach Stage I or early Stage II of the behavioral changes brought about by apomorphine. This dose had to be established each day and ranged from 2.5 to 3.5 mg/kg. Stage 1 consisted of licking, energetic sniffing and poking of the nose into the interstices of a wire rat cage for a period of 5–30 min. Somewhat higher doses of drug caused biting of the cage wiring (Stage 2). A dose of 3–4 mg/kg IP was accompanied by intense biting of the cage wiring (Stage 3).

Catecholamine assay methods. Catecholamine analyses were performed by the hydroxyindole method of Hogans (see [19]). Briefly, tissues were homogenized in acidified butanol and aliquots were added to a phosphate buffer (0.1 M, pH 6.5) for extraction of catecholamines. Iodine oxidation was used for conversion of the NE and DA to the respective trihydroxy- and dihydroxy- indoles. Samples containing NE were read in an Aminco-Bowman spectrophotofluorometer at 385/485 nm (activation/emission) wavelengths (uncorrected), while samples containing DA were read at 320/380 nm (uncorrected).

Statistical analysis. Significance of the levels of NE 1 hr or 24 hr after the last hormonal treatment was obtained after the use of Dunnett's test for comparison of the controls with the animals treated with MIF-I or MSH. Preliminary investigation of the decline in NE or DA values after AMPT was carried out by the analysis of variance and Scheffé's test to extract the effect of saline controls vs. MIF-I or MSH on the rates of NE decline for the intervals 0–2, 0–4, 4–6, and 0–6 hr. Linear regression analyses were also attempted after the data were logarithmically transformed, and the slope of the regression plus its standard error was determined [1,12]. Results of the apomorphine stereotypy experiment were evaluated by Fisher's Exact Probability Test.

RESULTS

Effect of MIF-I and MSH on Endogenous Levels of Catecholamines in Rat Brain

The levels of NE in various brain regions of intact and hypophysectomized rats were determined 24 hrs after final treatment with MSH or MIF-I (Table 1) and in another set of experiments, within 1 hr after the last hormonal treatment (Table 2). As seen in Table 1, MIF-I and MSH significantly decreased NE levels in the hypothalamus by 12 and 9 percent respectively. It was also found that MSH increased the hippocampal levels of NE by 10 percent. However, neither MSH nor MIF-I altered the resting levels of catecholamines in the striatum or midbrain 24 hr after the AMPT injections. Table 2 records additional NE levels for both unoperated and hypox rats. These samples were taken within 1 hr after AMPT injection from the areas examined and recorded as 0 min. NE levels for larger experiments attempted to examine the effects of MIF-I and MSH on the disappearance of endogenous catecholamines. The AMPT was used as a tyrosine hydroxylase inhibitor to prevent DA and NE synthesis. It can be seen that a 27 percent decrease in hippocampal levels of NE was found for intact rats only after administration of MIF-I. A small non-significant change in NE levels was recorded in the hippocampal region of hypox rats after MIF-I injections. The small but statistically significant ($p<0.05$) decrease in hypothalamic NE levels of unoperated rats seen 24 hrs (Table 1) after the last injection of MIF-I was not yet obvious 1 hr (Table 2) after the injection. A small drop of 11–12 percent in NE

TABLE 1

EFFECTS OF α-MELANOCYTE STIMULATING HORMONE (MSH)[+] AND MSH-RELEASE INHIBITING FACTOR (MIF-I)[+] ON CATECHOLAMINE CONTENT OF VARIOUS REGIONS OF THE BRAIN OF UNOPERATED RATS

Treatment	(NE) Pons-Medulla	(NE) Midbrain	(NE) Hypothalamus	(NE) Frontal Cortex	(NE) Hippocampus	(NE) Cerebellum	(DA) Striatum
Sal x 3	0.79 ± .03(5)[‡]	0.61 ± .03(5)	2.05 ± .04(16)	0.42 ± .02(5)	0.38 ± .01(16)	0.21 ± .01(11)	9.75 ± .21(4)
MIF-I x 1	0.74 ± .01(2)	0.63 ± .01(2)	1.92 ± .05(13)	0.39 ± .01(3)	0.37 ± .01(15)	0.22 ± .01(9)	9.15 ± .39(3)
MIF-I x 3	0.80 ± .03(5)	0.60 ± .03(5)	1.80 ± .07(16)**	0.42 ± .01(5)	0.41 ± .01(17)	0.19 ± .01(9)	9.15 ± .39(5)
α-MSH x 3	0.80 ± .01(5)	0.57 ± .04(5)	1.87 ± .09(9)*	0.43 ± .02(5)	0.42 ± .01(7)*	0.19 ± .01(10)	9.75 ± .21(4)
α-MSH x 1	0.83 ± .04(3)	0.60 ± .01(3)	1.88 ± .06(14)*	0.41 ± .02(5)	0.39 ± .02(13)	0.21 ± .01(3)	9.75 ± .14(3)

[+]MIF-I (20 mg/kg i.p.) and α-MSH (100 μg/kg i.p.) were administered at 24 hr intervals in groups that received three equimolar injections. All animals were sacrificed 24 hr after final treatment.

[‡]Number of animals is indicated in parentheses.

*P < .05

**P < .01

TABLE 2

EFFECT OF MIF-I AND α-MSH ON CATECHOLAMINE LEVELS IN VARIOUS REGIONS OF INTACT AND HYPOPHYSECTOMIZED RATS

		Brain Region (NE or DA in μg/g ± SEM)						
Animal	Treatment	NE Pons-Medulla	NE Midbrain	NE Hypothalamus	NE Frontal Cortex	NE Posterior Cortex	NE Hippocampus	DA Striatum
Intact	Saline	0.72 ± .02(6)[b]	0.58 ± .02(10)	2.21 ± .09(10)	0.34 ± .02(6)		0.52 ± .03(6)	8.60 ± .08(8)
	MIF-I	0.71 ± .02(7)	0.52 ± .02(10)*	2.18 ± .07(11)	0.36 ± .02(6)		0.38 ± .02(6)**	8.59 ± .16 (10)
	α-MSH	0.75 ± .02(6)	0.61 ± .02(10)	2.34 ± .08(9)	0.32 ± .02(6)		0.51 ± .03(6)	8.39 ± .08(8)
Hypophysectomized	Saline	0.64 ± .02(8)	0.52 ± .02(8)	2.09 ± .11(8)	0.22 ± .01(8)	0.22 ± .01(8)	0.46 ± .01(8)	9.71 ± .40(8)
	MIF-I	0.61 ± .02(7)	0.46 ± .01(7)*	1.81 ± .06(7)	0.29 ± .01(7)	0.21 ± .01(7)	0.42 ± .02(7)	8.15 ± .25(7)**
	α-MSH	0.55 ± .01(8)**	0.47 ± .01(8)*	1.85 ± .04(8)	0.31 ± .01(8)	0.25 ± .01(8)*	0.49 ± .03(8)	9.09 ± .48(7)

[a] Animals were sacrificed 15 min after α-MSH and saline; or 60 min after MIF-I

[b] Numbers in parentheses indicates the number of animals per group

* Indicates significant difference from control, $p < 0.05$.

** Indicates significant difference from control, $p < 0.01$

levels found in the mid-brain 1 hr after the last injection of MIF-I for both unoperated and hypox animals was significant ($p<0.05$) (Table 2). Other brain areas affected by either of the peptides employed were the posterior cortex (14 percent decrease after MSH, $p<0.05$), the striatum (16 percent decrease in DA after MIF-I, $p<0.01$) and the pons-medulla (15 percent decrease in NE after MSH, $p<0.01$).

Effects of MIF-I and MSH on Catecholamine Disappearance From the Brain After Treatment of Intact Rats with AMPT

Analysis of variance was applied to the 0, 1, 2, 4 and 6 hr values for catecholamines under the conditions listed in the Method section. Significant changes in the rate of NE disappearance were found only in the mid-brain region of rats treated either with MSH or MIF-I. It was noted that the rate of decline in NE levels of the MSH-treated animals was significantly greater than that obtained in the control group for the 0–6 hr interval, $F(14,132) = 2.63$, $p<0.01$. As shown in Fig. 1, the greatest difference was observed in the last 2 hr of the experiment. A comparison of the disappearance of NE for the controls vs the animals treated with MIF-I (Fig. 1), reveals that the overall disappearance rate for 0–6 hr was approximately the same for both groups, $F(14,132) = 1.79$. However, after treatment with MIF-I, the rate of decline in NE levels was less than that for the controls during the initial 4 hr of the experiment, $F(14,132) = 9.18$, $p<0.01$, but greater than the controls during the succeeding 2 hr period, $F(14,132) = 2.86$,

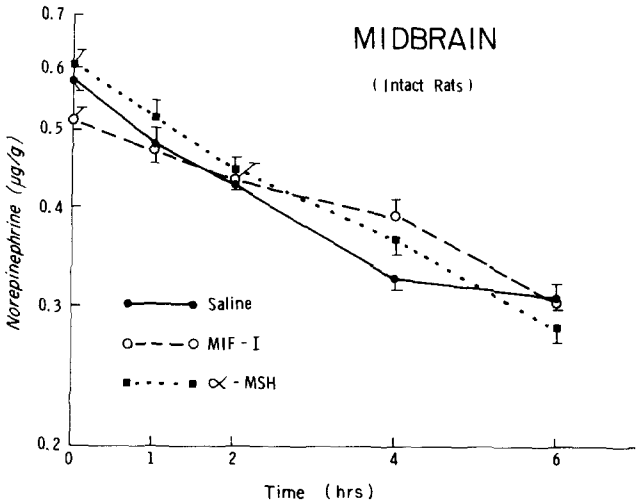

FIG. 1. Effect of MSH and MIF-I on turnover of NE in midbrain of intact rats. Ordinate indicates tissue concentration of NE. Abscissa indicates time of sacrifice of animals after AMPT administration (300 mg/kg IP).Each point is the mean ± SEM of 8 to 10 animals.

$p<0.01$. A further Scheffé analysis of the rates for the 0–4 and 4–6 hr periods also indicates a significant difference in these rates of NE disappearance between the two time periods. In the striatum of intact rats, no significant changes in the rates of DA disappearance were noted between the treated and the control groups after the injection of either MSH or MIF-I.

Effects of MIF-I and MSH on Catecholamine Disappearance From the Brain After Treatment of Hypox Rats with AMPT

These experiments were performed in exactly the same manner as those for the unoperated rats. Significant changes in rates of disappearance of catecholamines were found in the mid-brain, hypothalamus, pons-medulla, frontal cortex and striatum, after either MIF-I or MSH.

In the mid-brain MSH significantly decreased the rate of disappearance of NE during the 0–6 hr interval, $F(14,106) = 4.42$, $p<0.01$, with the greatest difference occurring during the final 4 hr (Fig. 2). This significant change is, however, diametrically opposed to the disappearance of NE found for unoperated animals where an increase in the disappearance rate was seen during the last 2 hr period. Also in the mid-brain area MIF-I decreased the rate of disappearance of NE during the initial 4 hr period after the injection of AMPT and stimulated the disappearance of NE during the last 2 hr of the study, $F(14,109) = 5.89$, $p<0.001$, as it did with the intact rats. Analysis of variance indicates, however, that the overall turnover of NE was not modified by MIF-I, as was also the case for the unoperated group of rats treated with MIF-I.

Although NE disappearance in the hypothalamus was not altered by MIF-I and MSH in intact rats, both treatments were found to decrease the decline in NE in the hypothalamus of hypox rats in the first 4 hr after AMPT injection (Fig. 3). Both the analysis of variance and the Scheffé test indicate significant differences after MIF-I, $F(14,106) = 2.09$, $p<0.01$, and MSH $F(14,106) = 3.20$, $p<0.01$.

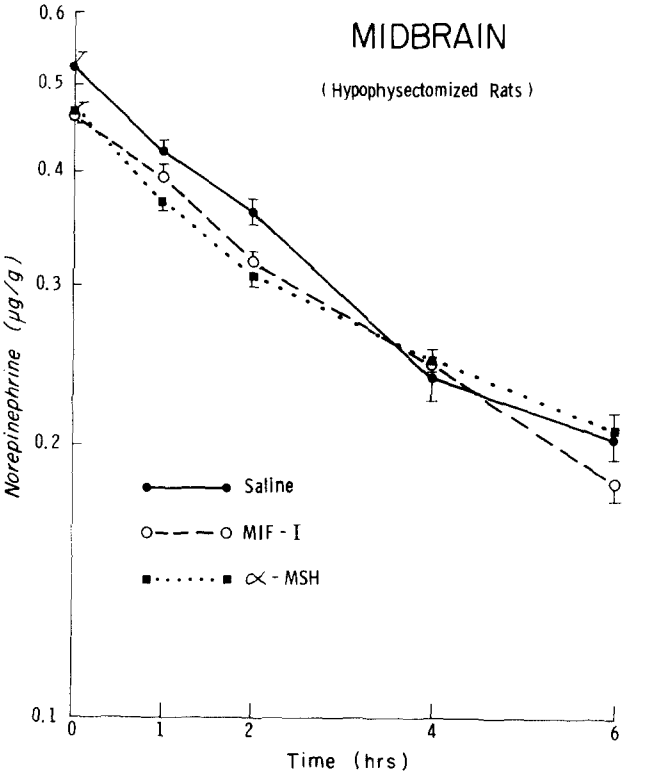

FIG. 2. Effect of MSH and MIF-I on turnover of NE in midbrain of hypophysectomized rats. Legend as in Fig. 1.

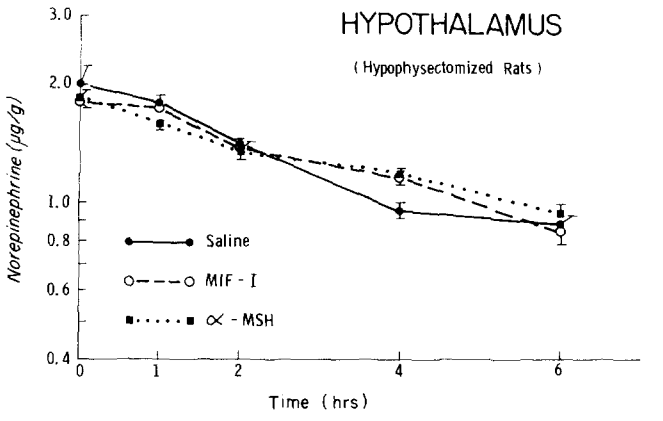

FIG. 3. Effect of MSH and MIF-I on turnover of NE in hypothalamus of hypophysectomized rats. Legend as in Fig. 1.

The graphs for the disappearance of NE in the pons-medulla (Fig. 4) and the frontal cortex (Fig. 5) have also been included because an analysis of variance on NE levels in the 0–6 hr period indicates a small but distinctly positive overall interaction. However, it should be noted that Scheffé tests did not indicate a significant change in the rates of NE decline after MSH and MIF-I so that the significance of the analysis of variance is brought into question in this case.

In the striatum of hypox rats, it was found that MSH significantly decreased the decline in DA levels over the 6 hr

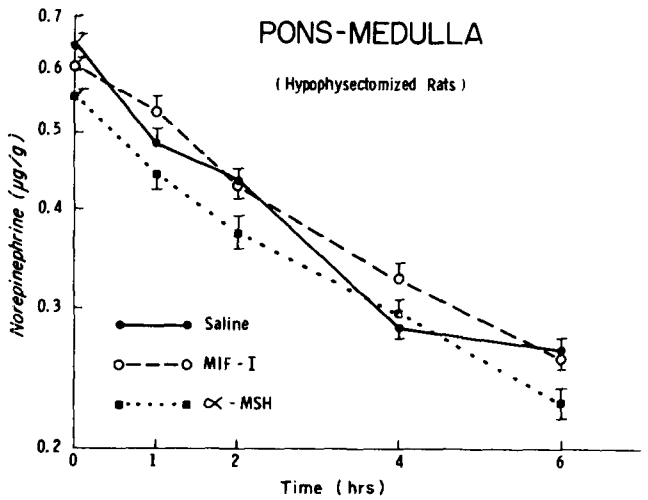

FIG. 4. Effect of MSH and MIF-I on turnover of NE in pons-medulla of hypophysectomized rats. Legend as in Fig. 1.

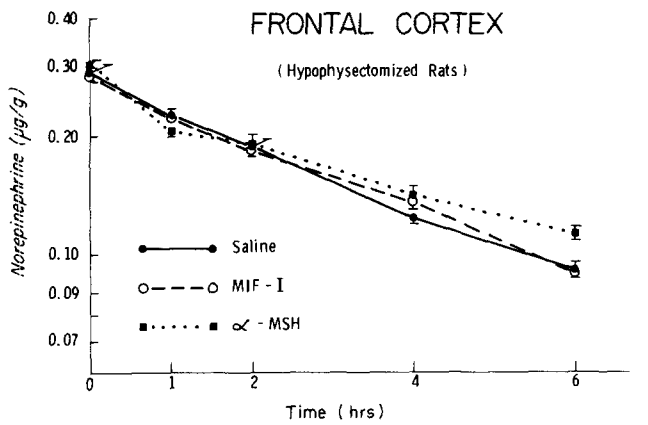

FIG. 5. Effect of MSH and MIF-I on turnover of NE in neocortex of hypophysectomized rats. Legend as in Fig. 1.

period of study (Fig. 6). MIF-I similarly decreased the breakdown of DA during the initial 2 hr period, although the overall decrease in DA was not modified by MIF-I according to the analysis of variance test.

Effect of MIF-I and MSH on Apomorphine Stereotypy

A control group of rats was injected daily with apomorphine to establish the dosage necessary to reach Stage 1 or early Stage 2 stereotypic activity (see Part C of Method Section). The stages were independently evaluated by 2–4 observers. Two other groups of rats were pretreated with either MIF-I (20 mg/kg, IP) or MSH (100 μg/kg, IP). Other controls used were animals which received MSH or MIF-I beforehand, but only saline instead of apomorphine later. Additional controls consisted of rats receiving saline instead of peptide prior to apomorphine treatment. A total of 120 rats was used in this study. For rats receiving sufficient apomorphine to attain Stage 1 only, pretreatment with either MSH or MIF-I never brought a singificant number to Stages 2 or 3. For similar groups of rats receiving enough apomorphine to reach early Stage 2 of Ernst's

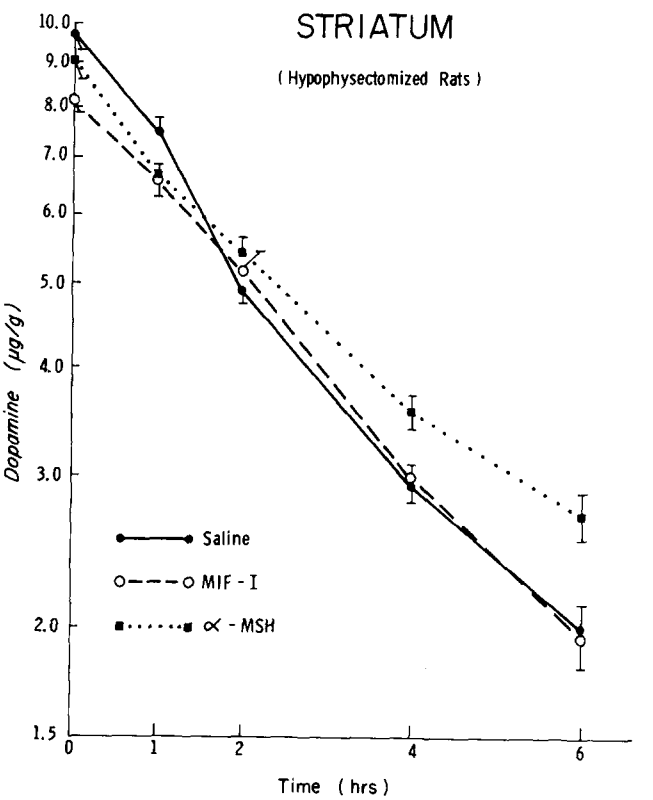

FIG. 6. Effect of MSH and MIF-I on turnover of DA in the striatum of hypophysectomized rats. Legend as in Fig. 1.

classification, neither peptide caused a greater number of rats to descend to Stage 1 or to ascent to Stage 3.

DISCUSSION

During the study of NE disappearance after injection of AMPT, it became obvious that the rate of decline in NE was not always linear during the 6 hr test period in intact and in hypox animals. Some support for this observation had been reported by Glowinski *et al.* [11] in their discussion of the method of Brodie *et al.* [1] for studying the application of steady state kinetics for the estimation of synthesis rate and turnover time of brain catecholamines. Whereas Brodie *et al.* [1] postulated a simple exponential decrease in NE disappearance for at least 6 hr, Glowinski *et al.* [11] indicated that a two-phase process took place, an initial faster rate of disappearance lasting perhaps an hour followed by a slower rate lasting for several hours. The faster disappearance rate, supposedly corresponded to the release of a rapidly used pool, followed by the breakdown of a more stable (storage) pool.

In our experiments we were able to corroborate the work of Glowinski to some extent except that the rate changes were not so simple (Figs. 1–6). Many times we found a somewhat faster rate of NE disappearance during the first 2 hr of our experiments. Often, however, a relatively linear rate lasted for 4 or even 6 hr.

Analysis of linear regression lines with the smallest error terms over 0–2, 0–4, or 0–6 hr periods often revealed rate differences in each brain region for each of the different periods. Also, in the same tissue region, control lines would often break at different times than the lines for the

hormonally treated groups. This would result in different rate constants, depending on whether, for example, 0–1 was compared with 0–2, or 0–4 compared with 2–6 in the same figure. We, therefore, resorted to an analysis of variance which gave us an idea of overall significance between the controls and all the hormonally treated groups, followed by a Scheffé analysis of the individual comparisons of a control with one hormonally treated group. Various time periods could also be extracted and analyzed. The figures showing the means of NE levels at each time period gave the information as to the direction of the significant rate changes.

The lack of an effect of MIF-I on steady state levels of endogenous DA in the striatum of intact animals in the present experiments is in direct contrast to the report of Friedman *et al.* [9], but in agreement with the finding of Plotnikoff *et al.* [25]. Although we found that DA levels among the hypox animals were decreased by 16 percent, it was not possible to show any alterations in DA levels for both unoperated and hypox animals. Therefore, no correlation between alterations in DA levels and accompanying behavioral changes could be made.

When the DOPA potentiation test [21] was performed with unoperated animals in the presence of MIF-I, it was found that this hormone doubled the amount of DA formed from the exogenously added d,l DOPA 30 min after the injection of the latter [30]. Why endogenous DA formation in unoperated rats should not be affected by MIF-I whereas DA formation after the injection of exogenous d,l DOPA should double after MIF-I is not known at present. Likewise, it is not clear why neither MIF-I nor MSH influences behavior brought about by an injection of apomorphine, when animals pretreated with d,l DOPA to study DA formation are affected by MIF-I. Perhaps one of the explanations for these findings is that MIF-I exerts its effect on DOPA-decarboxylase and not directly on the DA receptor where apomorphine supposedly acts.

Our study of the rates of disappearance of DA was performed in a similar manner to the study of NE disappearance. AMPT was injected to inhibit tyrosine hydroxylase and thus the synthesis of DA. The disappearance of the DA present was then measured at different time periods to establish a rate. Friedman *et al.* [9] measured the in vitro synthesis of ^{3}H-DA from ^{3}H-tyrosine in slices from the striatal brain area of a rat. It is however uncertain whether accurate rate measurements can be obtained under their conditions without at the same time measuring the specific activity of a metabolic precursor of DA, preferably DOPA, which is formed from tyrosine by the rate limiting enzyme tyrosine hydroxylase. This follows from the fact that it is dangerous to assume that intermediary metabolites have the same size pool in each group of animals or in tissue slices from different groups of animals (non-hormonally treated as opposed to hormonally treated groups). Thus, even if the same concentration and sepcific activity of ^{3}H-tyrosine and the same rate of tyrosine hydroxylase activity were present, the specific activity of the DOPA formed could be different if the pool size of DOPA in the controls were significantly different from that of the hormone-treated animals. Variable specific activities of DOPA could lead to different DA specific activities, even in the presence of an unchanged DOPA-decarboxylase activity or unchanged DA pools. Different DA pools would complicate the interpretation of such data even more. Even if the results of Friedman *et al.* indicating that MIF-I altered the rate of DA synthesis in intact rats were accepted, the fact that these investigators found no changed DA synthesis rate for hypox animals after addition of exogenous tyrosine would preclude the possibility of a correlation between their DA synthesis data and the change in behavior brought about by the injection of exogenous DOPA in intact and hypox rats [9].

Leonard [18] never reported on changes in NE turnover occurring after $ACTH_{4\text{-}10}$ and $ACTH_{1\text{-}10}$ in hypox animals. He did claim that $ACTH_{4\text{-}10}$ and $ACTH_{1\text{-}10}$ altered the turnover of NE in most areas of the brains of intact rats he investigated, but only $ACTH_{4\text{-}10}$ altered it in the mid-brain. However, a perusal of his data appears to indicate that the tests used for statistical significance between the controls and the hormonally treated animals were only performed on NE levels found 3 hr after the start of the experiments and therefore do not represent changes in rate.

The neurochemical changes we found after treatment of intact control rats with MSH are somewhat at variance with findings of Versteeg [33,34]. He used $ACTH_{4\text{-}10}$ or $ACTH_{1\text{-}10}$, fragments of ACTH with amino-acid sequences like those found in MSH and noted a 24 percent increase of the NE disappearance in the brain stem of intact rats. In roughly equivalent experiments performed by us, the disappearance rate of NE in animals pretreated with AMPT was measured assuming that NE disappearance would not be altered by this tyrosine hydroxylase inhibitor. We then subdivided the brainstem into hypothalamus, pons-medulla and mid-brain. Of these three regions, the rate of NE disappearance was altered by MSH only in the mid-brain, where it initially was unchanged and then increased at 4–6 hr. In addition, we observed a lower rate of NE disappearance in hypox rats for which Versteeg [33] had noted no differences from controls. Therefore, Versteeg's data did not allow for a correlation of his neurotransmitter changes with the behavioral changes expected to appear in both types of animals. It is also doubtful whether our data allow for such a correlation, since behavior was altered in the same direction for both unoperated and hypox rats while the neurotransmitter changes were in opposite directions as mentioned above (Figs. 1 and 2). Such was not the case for MIF-I where it can be seen (Figs. 1 and 2) that the changes were identical for both types of rats. These changes were accompanied by an 11–12 percent fall in 0 min NE levels for all the rats involved in these experiments (Table 2). From the same table it can be seen that, for rats treated with MSH, a lowering of NE levels occurred only for hypox rats.

It should be emphasized that finding an altered rate of NE disappearance together with behavioral changes after treatment of intact and hypox rats with MSH and MIF-I does not necessarily imply a causal relationship between them. NE levels could be unchanged with unaltered, depressed, or increased rates of NE disappearance. It is also possible that differences in the disappearance of NE are completely unrelated to behavioral changes. Many of our findings with serotonin [31], DA, and NE support this possibility. Hormonal influences could be directed towards alteration of neuronal membrane receptors, so that a given amount of NE could cause a shift in the resting membrane potential. Thus, it is conceivable that the hypothalamic

hormones bring about their presumed changes by altering the cyclic nucleotide content of the postsynaptic cell membrane receptors which in turn could alter the ionic permeability of the membrane and thus the state of polarization and ability to relay impulses.

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