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Investigations on α-MSH and MIF-I Effects on Cyclic AMP Levels in Rat Brain[1]

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It has been suggested that the peptides alpha-melanocyte stimulating hormone (α-MSH) and MSH-release inhibitory factor (MIF-I) may alter adenosine-3′, 5′-cyclic monophosphate (cAMP) metabolism [13,26]. Normal and hypophysectomized (hypoxed) rats were administered saline (controls IP daily × 3), α-MSH (80 μg/kg IP daily × 3) or MIF-I (1 or 10 mg/kg IP daily × 3) and sacrificed 30 min after the third injection in a focused microwave oven (1.5 KW; 2–3 sec). Various brain areas were then assayed for cAMP levels after each treatment. The occipital cortex area was the only area to show consistent changes in both normal and hypoxed rats after α-MSH treatment. These findings were replicated for the occipital cortex in a second group of normal and hypoxed rats which were similarly treated. The results suggest a correlation between the rise in cAMP found and reported changes in visual acuity and attention in rats and humans after treatment with α-MSH [8, 14, 23].

α-MSH MIF-I Cyclic AMP Rat brain

IN man and lower mammals, there are no known physiological functions for the putuitary hormone melanocyte stimulating hormone (α-MSH) or for the hypothalamic factor (MIF-I) which inhibits its release in some assay systems. Both peptides, however, are present in most animals from amphibians up to and including man. Despite the lack of proven physiological functions for these naturally occurring peptides, numerous reports concerning significant behavioral and electrophysiological changes have appeared after their administration to man and lower mammals. The many reports concerning the effects of α-MSH on various behavioral paradigms in both albino and pigmented rats as well as intact and hypophysectomized (hypoxed) rats have been summarized and reviewed elsewhere [9,12]. In addition, Kastin *et al.* [8], Sandman *et al.* [24] and Miller *et al.* [14] have reported increased visual acuity and attention and increased somatosensory evoked responses in men administered α-MSH or ACTH/MSH 4–10. Furthermore, significant changes were observed in electroencephalographic (EEG) recordings from human subjects who had been administered α-MSH or ACTH/MSH 4–10.

The reported behavioral effects of α-MSH and the numerous reports with respect to MIF-I on several behavioral tests [7, 18, 19, 20] led to the postulate that these behavioral changes might be associated with concomitant neurotransmitter changes [21,27]. Spirtes and his coworkers then investigated the effects of these peptides on the levels and rate of metabolism of serotonin (5-HT), dopamine (DA) and norepinephrine (NE) [13,25]. No changes were found by them in striatal DA levels or DA disappearance in in vivo studies using normal rats administered MIF-I. A significant decrease in striatal DA levels was found in hypoxed rats which had received 3 injections of MIF-I intraperitoneally (IP) over a 72 hour period. In addition, there was a significant decrease in the rate of decline of DA levels for both α-MSH and MIF-I in the presence of α-methyl paratyrosine in hypoxed rats. Similarly, with respect to NE disappearance rates, α-MSH produced a significant decrease in the rate of NE disappearance in the midbrain area in hypoxed rats whereas in the intact rats the rate of disappearance was greater. The overall disappearance of NE at the end of a 6 hour period was not affected by MIF-I in either hypoxed or intact rats [13]. Kostrzewa *et al.* also reported that the peptides affected the rate of disappearance of NE from several other brain areas; however, the effects occurred only in the hypoxed rats and not the normal rats. In view of the fact that the above

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mentioned neurotransmitter changes may not be the direct cause of the behavioral alterations, Kostrzewa *et al.* suggested that the peptides (α-MSH, MIF-I) affected cyclic nucleotides directly via post synaptic membrane receptors. Numerous reports in the literature have suggested that α-MSH activates adenylate cyclase thus increasing adenosine-3',5'-cyclic monophosphate (cAMP) in frog and reptilian skin [1, 6, 16, 17]. We therefore decided to study the effects of α-MSH and MIF-I on the levels of cAMP in various brain areas of normal and hypoxed rats.

METHOD

Twenty four normal male Sprague-Dawley rats and 24 hypoxed male Sprague-Dawley rats (125–150 g from Charles River Labs, Wilmington, MA) were used for all experiments. Control rats were administered saline (0.9%)-ascorbic acid (0.1%). Peptide treated rats were administered α-MSH (10^7 U/mg, 80 μ/kg) or MIF-I Pro-Leu-Gly-NH_2, (1 or 10 mg/kg) at 24 hr intervals for a total of 3 injections. Both α-MSH and MIF-I were dissolved in the saline-ascorbic acid solution used in the rats considered controls. All doses were administered IP in less than 0.75 ml. Thirty minutes after the third injection the rats were sacrificed in a specially modified Hobart 12501. microwave oven (1.5 kW). The animals were exposed to the focused microwave radiation (MWR) for 2½–3 sec, which was sufficient to raise the brain temperature to 85–90°C and thus inactivate all cyclic nucleotide metabolic enzymes. After the exposure to the MWR, the rats were decapitated and their brains removed and dissected by the procedure of Glowinski and Iversen [4] with the following modifications: the midbrain area was subdivided into the thalamus and midbrain by extending the posterior and lateral borders for the hypothalamic area dorsal to the level of the lateral ventricles. This piece was then hemisected horizontally at the level of the anterior commissure for the hypothalamic area ventrally and the thalamic area dorsally. In addition, the posterior third of the neocortex section was removed and labeled as the occipital cortex region. Immediately after dissecting each area, it was wrapped in aluminum foil, frozen on dry ice, and stored at −50°C until assayed. For the cyclic nucleotide extractions, the tissues were rapidly weighed (within 10 sec) and homogenized in 10% trichloracetic acid (10% TCA) at 4°C. They were then centrifuged at 5,000 × g (maximum) for 20 min, the supernatant removed and the TCA extracted with 4–5 volumes of water-saturated diethyl ether. The ether extraction consisted of layering 4–5 volumes of ether onto the TCA-aqueous layer and vigorous shaking for 20 sec by means of a vortex shaker. This was repeated 5 times, with the final shaking lasting for a duration of 60 sec. The samples were then equally divided and dried using a Buchler Evapomix, after which they were capped with parafilm and stored at −20°C until assayed for cAMP. The cAMP assay employed is that of Gilman [3] using a cAMP binding protein isolated from bovine skeletal muscle as described by Miyamoto *et al.* [15]. The data for each assay were entered into a preprogrammed Hewlett-Packard (Model 9820A) desk top computer which plotted the correlation of regression for the standard curve and automatically determined the number of picomoles (pM) of cAMP and the number of pM/mg wet weight of tissue for each individual sample.

The experimental data were analyzed for the difference of the means using an analysis of variance on a $\log_{10}$ of the skewed data. Duncan's New Multiple Range (DNMR) test was then used to compare the control (saline) and treated groups (α-MSH and MIF-I).

RESULTS

Table 1 presents the changes observed in cAMP levels in the frontal cortex, striatum, midbrain, thalamus, parietal and occipital cortical areas for both normal and hypoxed rats. The analysis of variance showed a significant three way interaction of treatment (i.e. normal or hypoxed) vs peptides vs areas ($F_{15,200} = 3.11$, $p < 0.0003$). In addition, subsequent DNMR test indicated there were significant ($p < 0.05$) increases in cAMP levels after MIF-I (1 mg/kg) in the intact parietal cortex and with α-MSH and MIF-I (10 mg/kg) in the intact occipital cortex. Furthermore, α-MSH produced a highly significant ($p < 0.01$) increase in the level of cAMP in the midbrain of intact rats whereas in the striatum there was a significant ($p < 0.05$) decrease in cAMP after α-MSH. The hypoxed rats showed a highly significant ($p < 0.01$) increase in cAMP in midbrain and thalamus after

TABLE 1

EFFECTS OF α MSH AND MIF-I ON cAMP LEVELS IN VARIOUS BRAIN REGIONS OF INTACT AND HYPOPHYSECTOMIZED RATS

Animal	Treatment	Front. Cx.	Prtl. Cx.	Occip. Cx.	Midbrain	Striatum	Thalamus
Intact	Saline x 3	1.55 ± 0.24	0.78 ± 0.09	0.32 ± 0.03	1.07 ± 0.11	1.08 ± 0.09	0.86 ± 0.07
	*α-MSH x 3	1.59 ± 0.15	1.03 ± 0.21	0.53 ± 0.08§	1.62 ± 0.20¶	0.77 ± 0.05§	0.99 ± 0.14
	†MIF-I x 3	1.44 ± 0.12	1.12 ± 0.21§	0.45 ± 0.08	0.89 ± 0.10	0.91 ± 0.12	0.85 ± 0.09
	‡MIF-I x 3	1.52 ± 0.12	0.84 ± 0.07	0.56 ± 0.08§	1.04 ± 0.11	0.83 ± 0.04	1.02 ± 0.12
Hypoxed	Saline x 3	1.64 ± 0.20	1.54 ± 0.16	0.99 ± 0.25	1.18 ± 0.20	0.86 ± 0.09	0.95 ± 0.17
	*α-MSH x 3	1.97 ± 0.13	1.35 ± 0.11	1.44 ± 0.18§	1.35 ± 0.15	1.02 ± 0.08	0.87 ± 0.13
	†MIF-I x 3	1.53 ± 0.15	1.70 ± 0.12	0.90 ± 0.06	1.90 ± 0.27¶	1.02 ± 0.06	1.68 ± 0.13¶
	‡MIF-I x 3	1.80 ± 0.12	1.77 ± 0.29	1.15 ± 0.24	1.39 ± 0.04	0.93 ± 0.06	0.98 ± 0.18

n = 6 rats/group.
*Dose 80 μg/kg IP every 24 hr.
†Dose 1 mg/kg IP every 24 hr.
‡Dose 10 mg/kg IP every 24 hr.
§Indicates significant difference from control (saline) $p < 0.05$.
¶Indicates significant difference from control (saline) $p < 0.01$.
All cAMP values are pM/mg wet tissue.

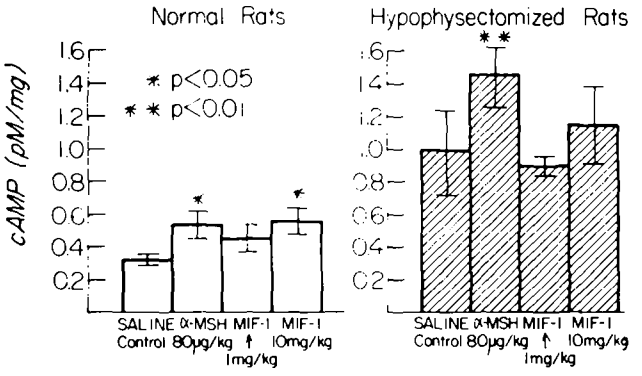

FIG. 1. Effect of α-MSH and MIF-I on cAMP levels in occipital cortex of first group of rats. All rats were sacrificed 30 min after the third injection by exposure to focused microwave radiation (1.5 KW, 2–3 sec). Values are mean cAMP ± standard error of mean.

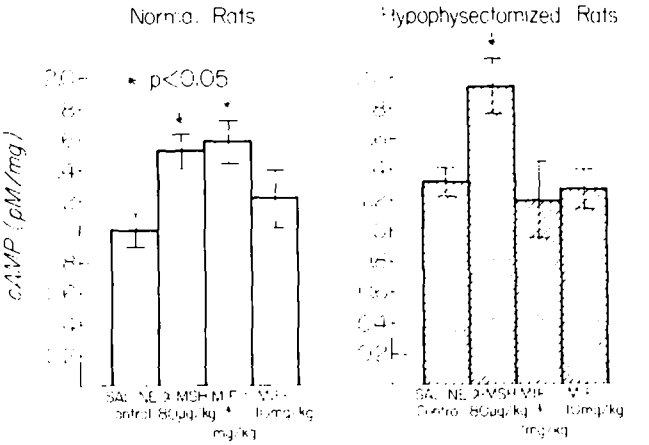

FIG. 2. Effect of α-MSH and MIF-I on cAMP levels in occipital cortex of second group of rats. These studies were a replication of the initial findings in Fig. 1.

injections of MIF-I (1 mg/kg) and a significant increase in cAMP levels in the occipital cortex after α-MSH injection. The only changes which were consistent in both intact and hypoxed rats were the changes in the occipital cortex following α-MSH treatment (see Fig. 1). In order to verify the changes in cAMP levels in the occipital cortex, these experiments were replicated using another group of 24 intact and 24 hypoxed rats and only the changes in this area were followed. The results are presented in Fig. 2 and can be seen to be essentially the same. Significant ($p<0.05$) increases in cAMP levels were observed for both intact and hypoxed rats in the occipital cortex.

DISCUSSION

The analysis of variance and DNMR tests indicated that the peptides produced significant changes in some brain areas [parietal cortex from intact rats after MIF-I (1 mg/kg); occipital cortex from intact rats after MIF-I (10 mg/kg); midbrain and striatum from intact rats after α-MSH; midbrain from hypoxed rats after MIF-I (1 mg/kg); thalamus from hypoxed rats after MIF-I (10 mg/kg)]. However, the fact that these changes were not observed in both groups (normal and hypoxed rats) after treatment with either peptide indicates that such changes may not be direct cerebral effects.

However, there were significant ($p<0.05$) changes in cAMP levels in occipital cortex of both intact and hypoxed rats after treatment with α-MSH. This correlates well with previous reported findings of Sandman *et al.* [22], Kastin *et al.* [8] and Miller *et al.* [14]. They reported increased visual acuity and attention in both experimental animals and human subjects after the administration of α-MSH or ACTH/MSH 4–10. In the present experiments the fact that the results could be replicated with a second group of animals demonstrates the reliability of the findings. Moreover, blood flow in the rat occipital cortex after administration of α-MSH is not reduced as it is in other areas of the brain [5]. In addition, ^{3}H-α-MSH has been shown to be localized in greater concentration in the occipital cortex than elsewhere after intracarotid injection [10]. It has long been established that the occipital cortex is the primary visual association area in the neocortex [2] and it now appears that α-MSH may affect this area directly. It is, however, also possible that αMSH may influence other points along the visual pathway, as for example the retina itself. Consequently, it is possible that α-MSH acts upon the rods in the retina and directly alter the visual pigment rhodopsin, although no experimental proof is at hand for such a phenomenon.

The increase in cAMP in the occipital cortex may be correlated to the increase in local and somatosensory evoked potentials found in animals and humans beings administered α-MSH [8, 12, 14]. Furthermore, the changes observed in lower vertebrates with respect to skin melanocytes are known to serve as a protective adaptation to help the animals blend with their surroundings. Quite possibly in these and other vertebrates as well, α-MSH serves as a protective adaptation by increasing visual acuity as a response to environmental changes.

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