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MSH Affects Regional Perfusion of the Brain[1]

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GOLDMAN, H., C. A. SANDMAN, A. J. KASTIN AND S. MURPHY. *MSH affects regional perfusion of the brain.* PHARMAC. BIOCHEM. BEHAV. 3(4) 661–664, 1975. – With the single exception of the occipital cortex, the flow of blood to most regions of the brains of conscious, unrestrained rats was reduced within 10 min after intravenous administration of αMSH. Though these effects were transitory for most regions of the brain, perfusion of cerebellum, pons and medulla, hippocampus, and parietal cortex was still significantly low by 20 min. Assuming that flow changes reflect functional changes, these early responses to αMSH suggest an explanation for the effects of this hormone in which visual learning is improved.

Melanocyte-stimulating hormone (MSH) Blood-flow Brain Occipital cortex

THE extrapigmentary effects of melanocyte stimulating hormone (MSH) occur most notably in the nervous system. These include electrophysiological alterations in a variety of animals, including man [3, 10, 12, 18]. MSH also inhibits the extinction of learned aversive [1,2] and appetitive behavior [19] and it has been suggested that endogenously secreted MSH may facilitate adaptive behavior by leading to increased attention and awareness of the environment [20]. Although limbic system functions have been ascribed to MSH [18], there are few clues to sites of action of MSH within the nervous system and few methods for surveying these sites.

As a means of screening various areas of the brain whose functions might be altered by hormones we have studied the regional perfusion of the nervous system in conscious, unrestrained rats after treatment with αMSH. The utility of this approach is based on the assumption that 1) functional and, therefore, metabolic activity determines in large part, the flow of blood to nervous tissues [4, 9, 13, 14, 23, 25] and 2) that functional changes elicited by hormones in various parts of the brain are sufficiently large so as to provoke changes in blood flow which are detectable by our method.

METHOD

A relatively convenient method, described in detail elsewhere [7] was used to measure simultaneously the flow of blood to each of 10 regions in the brains of conscious, unrestrained male rats. The method is our modification [7], of Sapirstein's indicator-fractionation technique [22]. Employing antipyrine – ^{14}C as the indicator, the flow-fraction of the cardiac output perfusing a region is mea-

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sured simultaneously with that of the cardiac output. The method, therefore, permits estimation not only of the distribution of the cardiac output but of the minimum absolute flow of blood which exchanges nutrients with the region, as well.

Animals and Procedure

The study was performed in male, Wistar rats, 75 to 83 days old; mean body weights at these ages ranged from 320 to 360 g, respectively. Three days before blood flow was measured, polyethylene catheters were implanted in one femoral vein and the opposite femoral artery. Our experience with another behavior-modifying hormone, estradiol-17β, which very quickly altered regional brain perfusion [24] suggested that MSH also might provoke a unique pattern of brain perfusion soon after administration. Consequently, on the day of measurement, synthetic αMSH (40 μg/kg – 1×10^7U/mg in 0.1 ml of 0.01 M acetic acid:0.9% saline) was administered through the venous catheter at 5, 10 or 20 min before measurement of flow. Twenty-two animals receiving saline and an additional 10 receiving the acetic acid:saline vehicle served as controls for the three time periods.

Flow measurements were accomplished by injecting intravenously a 40 μl bolus containing 1.5 μCi of antipyrine –^{14}C (26 μg); cardiac output was determined simultaneously by sampling the arterial contents of indicator. Small 100 μl arterial blood samples were collected before and after drug injections for blood-gas determinations. Animals were killed 20 sec after injection of indicator by rapid intravenous administration of 250 μl of a saturated KCl solution. Subcortical regions were dissected according to the protocol of Glowinski and Iversen [5]; telencephalic areas, described as frontal, parietal, and occipital, correspond to the parcellation of Krieg as Areas 10, 1–3, 17–18, respectively [11]. Tissue indicator was extracted (>98 percent) by the scintillation solvent (Bray's) and counted.

RESULTS

As expected, the ten animals treated with acetic acid: saline vehicle ($\leq$0.001 mEq HAc per animal) were unaffected on any measure – regional brain perfusion, cardiac output, blood gases or pH – at any of the time intervals examined as compared to the 22 controls treated only with saline. As a consequence, all values for these 32 animals have been combined and are listed as control values in Table 1.

The flow of blood to all areas of the brain, except the occipital region, decreased soon after intravenous administration of αMSH. Although recovery to near normal levels was evident by 20 min in most areas, flows to cerebellum, pons and medulla, midbrain, hippocampus and parietal cortex were still significantly low. Flow to the sole atypical region, the occipital cortex, was relatively constant throughout the 20 min interval following αMSH injection. The phasic changes in regional brain flow occurred in the face of a small but progressive decline in the cardiac output.

Arterial blood pH diminished slightly at 5 min and returned to control levels by 20 min. PCO_2 values, on the other hand, decreased slightly after 10 min and returned to control levels by 20 min. PO_2 values mirrored PCO_2 values, reaching a maximum at 10 min.

Between 5 and 10 min after administration of αMSH most animals were somewhat dystonic and when picked up they made few attempts to escape. Although appearing relaxed these animals were not drowsy; rather, they seemed attentive to nearby events.

DISCUSSION

It is obvious that the very small concentrations of αMSH which alter behavior [3, 18, 19, 21] also rapidly alter regional perfusion of the brains of conscious animals. The pattern is quite unlike others seen for estrogen [24] or potent drugs such as LSD [6], psilocybin and Δ^9-tetrahydrocannabinol (unpublished observations), ethanol [8], or pentobarbital [7] and onset of changes in flow were faster. Within 5 min most regions had lower flows, significantly decreased in the hypothalamus, hippocampus, and olfactory bulb and especially in subcortical areas involved with motor performance, the cerebellum and basal ganglia. By 10 min, flows to virtually all areas of the brain were depressed; with the exception of cortical areas, all subcortical regions received as little or less blood after αMSH treatment than after pentobarbital [7]. This was especially true for cerebellum and the brain stem which are resistant to the depressant actions of pentobarbital [7] and ethanol [8]. By 20 min, flow to most regions seemed to be returning to control levels with some exceptions, notably those of the cerebellum, pons and medulla, hippocampus and parietal cortex.

The decrease in plasma pH followed by a decreased PCO_2 and elevated PO_2, which was not seen in animals receiving the diluent vehicle, suggests that a transient metabolic acidosis followed administration of αMSH. Some of the decreased cerebral circulation may have resulted from the slight hypocarbia which, however, was too short-lasting to account for the effects of MSH on brain perfusion.

The mild dystonia observed in these animals when picked up was clearly not accompanied by drowsiness as indicated by Sakamoto [15]. It suggests rather a limited ability to perform coordinated muscular activity immediately following intravenous injection and for at least 10 min thereafter. Sandman, *et al.* [19] also have noted mild dystonic and erratic motor behavior in rats during certain learning situations. Such dystonic effects in rats are paralleled by the prolonged depressed perfusion of the cerebellum, which may be causally related.

Alpha MSH not only selectively affected regional flow but reduced its variability, as well; standard deviations for most regions of the brains of MSH treated animals were 50 to 60 percent less than for corresponding areas of control animals. This latter effect of MSH is not unique, however, for other behavior affecting drugs such as lysergic acid diethylamide – but not its inactive derivatives [6], Δ^9-tetrahydrocannabinol or psilocybin (unpublished observations) similarly reduce variability in regional perfusion. If flow indeed reflects function, then it is reasonable to expect that reduced variability of flow reflects more homogeneous behavior after drug treatment.

In contrast to all other regions of the brain, the perfusion of the visual cortex appeared to be uniquely resistent to the acute effects of αMSH. Since the perfusion of a brain area more than likely reflects its function [4, 9, 13, 14, 23, 25] the selective sparing of the occipital or visual areas of the cortex may explain the apparent visual attentiveness informally observed in this study. These findings also support our earlier speculations that MSH facilitated

TABLE 1

REGIONAL BRAIN BLOOD FLOW AS A FUNCTION OF TIME AFTER INTRAVENOUS INJECTION OF αMSH-40 μg/kg

Tissue	Control	5 Min	10 Min	20 Min
cerebellum	0.88 ± 0.02	0.81 ± 0.02*	0.80 ± 0.03†	0.81 ± 0.02†
pons & medulla	0.79 ± 0.02	0.73 ± 0.01	0.70 ± 0.02‡	0.72 ± 0.01†
hypothalamus	0.83 ± 0.01	0.77 ± 0.02*	0.72 ± 0.02[a]	0.78 ± 0.02
basal ganglia	0.84 ± 0.02	0.78 ± 0.02*	0.73 ± 0.02[a]	0.79 ± 0.02
midbrain	0.89 ± 0.01	0.83 ± 0.03	0.79 ± 0.02[a]	0.83 ± 0.02*
hippocampus	0.73 ± 0.01	0.67 ± 0.01†	0.63 ± 0.02[a]	0.65 ± 0.02§
olfactory bulb	0.75 ± 0.02	0.67 ± 0.02†	0.65 ± 0.02§	0.69 ± 0.02*
cortex				
frontal	0.97 ± 0.02	0.90 ± 0.02	0.86 ± 0.03§	0.90 ± 0.03
parietal	1.00 ± 0.02	0.93 ± 0.02	0.85 ± 0.02[a]	0.91 ± 0.02‡
occipital	1.02 ± 0.02	1.06 ± 0.02	0.98 ± 0.03	1.03 ± 0.02
cardiac output				
ml/min/kg	362 ± 12	338 ± 12	338 ± 12	314 ± 12†
arterial blood				
pH	7.42 ± 0.01	7.38 ± 0.01§	7.39 ± 0.01*	7.40 ± 0.01
P_{CO_2} mm Hg	41 ± 1	40 ± 1	37 ± 1‡	39 ± 1
P_{O_2} mm Hg	85 ± 2	86 ± 1	92 ± 1†	90 ± 2
number of animals	32	10	10	13

Blood flow is expressed in ml/min/g as means ± SE *$p<0.05$ †$p<0.025$ ‡$p<0.01$ §$p<0.005$ [a]$p<0.001$

information processing by increasing the visual attentiveness of animals tested in discrimination learning situations [16, 17, 21] and in man [10].

Our findings may help explain the hormonal and cerebrovascular dynamics of certain other classes of behavior. In addition to the relationship described above, we have found that conditions of stress and threat which result in narrowing or focussing of attention [26] are also related to endogenous release of MSH in rats [20]. Our data suggest that behavior, MSH release, selective perfusion and, presumably, function of certain brain regions may be causally related.

These results suggest a time-frame for future experiments; and help explain some aspects of past experiments, that is, that responses in learning situations are likely to be altered by early effects of MSH. In view of the rapid return of depressed perfusion to normal levels in many regions, there is the possibility also that some of the behavioral responses (especially long-term responses) may be consequences of recovery from, rather than direct effects of MSH.

Although the physiological substrates of behavior affected by MSH have yet to be completely described, the shifts in regional perfusion point to regions of the brain which appear to be unusually responsive to actions of this hormone. At this time, however, it is impossible to distinguish between primary sites and secondary functional aspects of MSH action.

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