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# Hormones and Regional Brain Blood Flow

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GOLDMAN, H., E. B. SKELLEY, C. A. SANDMAN, A. J. KASTIN AND S. MURPHY. *Hormones and regional brain blood flow*. PHARMAC. BIOCHEM. BEHAV. 5: SUPPL. 1, 165–169, 1976. – Acute effects of the hormones, estradiol-17$\beta$ and $\alpha$MSH, and of neonatal pretreatment with $\alpha$MSH on the flow of blood to regions of the brains of conscious adult rats have been determined with an indicator distribution technique. As previously reported, flows were reduced in most areas within 10 min after intravenous administration of $\alpha$MSH; only the occipital cortex was spared. Though these effects were transitory for most areas, perfusion of pons and medulla, cerebellum, hippocampus and parietal cortex was still low by 20 min. However, pretreatment with $\alpha$MSH during infancy led to persistent behavioral changes which were not accompanied by flow differences. Assuming that flow changes reflect functional changes, the rapid responses to $\alpha$MSH suggest an explanation for the effects of this hormone on visual learning and on the determination of subsequent learning behavior. By contrast, estradiol, within 10 min after injection, increased flow to most regions of the brain, especially the frontal cortex, hippocampus, basal ganglia and cerebellum; females were more affected than males. Flow changes were greater than those elicited by more obvious behavior-modifying drugs. Compared to $\alpha$MSH, the flow data for estradiol suggest a physiologic basis for a behavioral effect which is likely to be different yet, perhaps, equally profound.

$\alpha$MSH  Estradiol  Neonate  Regional brain blood flow  Occipital cortex  Frontal cortex

VARIOUS drugs which have specific effects on animal behavior uniquely redistribute the flow of blood to regions of the brain. For example, patterns of perfusion are qualitatively different for each of the hallucinogens, lysergic acid diethylamide (LSD) [9], $\Delta^9$-trans-tetrahydrocannabinol (THC) [8], and psilocybin (unpublished observations), as well as for the nervous system depressants, pentobarbital [6] and ethanol [7]. There is reason to believe that these alterations in flow reflect functional responses of unique and specific regions of the brain to these drugs. Of the drugs we have employed, however, the most potent in redistributing cerebral blood flow have been hormones, i.e., estrogen and MSH. We suspect that their effects on behavior, though not as obvious as those elicited by hallucinogens or depressants, are more profound.

This paper reports the continuing investigation of the effects of endocrine, and neuroendocrine substances on regional perfusion of the brain. It deals with the effects of acutely administered estradiol-17$\beta$ and a comparison of previous studies of acutely administered $\alpha$MSH with neonatally administered $\alpha$MSH. Some improvements in our methodology will also be described.

## METHOD

A relatively convenient method, described elsewhere [6] was used to simultaneously measure the blood flow to each of ten regions in the brains of conscious, unrestrained rats. The method is our modification of Sapirstein's indicator fractionation technique which can utilize any one of a variety of indicators such as $^{131}$I iodoantipyrine, $^{14}$C-antipyrine, or $^{14}$C-thiopental, which are able to enter the brain easily. The method permits estimation not only of the fractional distribution of the cardiac output but also the minimum absolute flow of blood which exchanges nutrients with a region, as well. The basic equation of the indicator-fractionation technique states that:

$$Ui/I = Fi/C.O.$$
$$\text{whenever } \int_0^T C a dt = \int_0^\infty C^i a dt \qquad [1]$$

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Where Ui = indicator uptake in tissue mass i at time (T)

I = body uptake of indicator

Fi = blood flow to tissue mass i

C.O. = cardiac output

$\int C'a$ = describes the indicator dilution curve obtained from the real one by substituting the extrapolated points after recirculation for the real ones

Equation [1] states, in effect, that when indicators such as antipyrine or thiopental are administered in a single intravenous injection and the killing time is short, then the pattern of their distribution in the brain will be the same as the pattern of the fractional distribution of the cardiac output.

*Animals*

The estrogen studies were carried out in male and four or five day cycling female Wistar rats, 68–87 days old. In the MSH experiment, infant male Holtzman albino rats were injected intraperitoneally with 10 μg synthetic αMSH ($1 \times 10^7$ U/mg), or diluent, daily from the second through the seventh day of life only. These otherwise normal rats were approximately 90 days old at the time of the measurement of blood flow and had been tested behaviorally only as adults.

*Procedure*

Three days before the measurement procedure was performed, PE-50 polyethylene catheters were implanted in one femoral vein and the opposite femoral artery. The blood vessel catheters were filled with heparin (1:1,000) and heat sealed; all catheters were brought up under the skin of the flank, the back, and the dorsal aspect of the neck, and stored in a covered plastic cap which was stitched in place. All cycling animals were cannulated on the first day after estrus and had leukocytic vaginal smears on the day of blood flow measurement.

On the day of the measurement, each animal was placed in a small black plastic box at $t = -20$ min and allowed a calming period of 10 min, after which the catheters were freed from the neck cap. The exteriorized ends of the catheters, brought through slits in the box, were long enough to provide some slack when the animal moved about in the box. At $t = -10$ min, isotonic-saline or estradiol-17β, 10 μg in 0.1 ml of a 0.5 percent ethanol-saline solution, was administered intravenously. At the same time a 100 μl blood sample was collected from the arterial catheter for pH and blood gas determinations in a standardized Radiometer BMS-3 blood gas machine. At $t = 0$, the label (1.5 μCi of $^{14}$C-antipyrine or $^{14}$C-thiopental) was flushed smoothly into the circulation with 120 μl of saline over a one sec period so as to minimize hemodynamic transients.

Also at $t = 0$, collection of one sec samples of arterial blood, about 15 μl each was begun for the determination of the cardiac output by an indicator-dilution technique [31] in which thiopental or antipyrine replaced the previously employed $^{86}$ rubidium indicator. Collection of arterial blood ended at $t = 15$ sec. At $t = 20$ sec, the animal was killed by a rapid, intravenous injection of 250 μl of a saturated KCl solution; the heart stopped within one beat.

Subcortical regions were dissected according to the protocol of Glowinski and Iversen [4]; telencephalic areas, described as frontal, postfrontal, parietal and occipital, correspond to the parcellation of Kreig as areas 10, 4–6, 1–3, 17–18, respectively [16]. Tissue indicator was extracted (>98%) by the toluene solvent in the case of thiopental, or Bray's solution in the case of antipyrine and counted.

An additional study was carried out in much the same way to establish the time-independency of indicator uptake in order to validate the use of thiopental to measure blood flow by the indicator-fractionation technique. In such studies, however, animals were killed from 10–60 sec after indicator injection and no arterial blood are collected. The content of thiopental in all brain areas examined was constant for at least 20 sec after injection. During this period, therefore, this indicator satisfied the essential time-independency requirement of the method and gave results which were similar to those obtained with antipyrine. Moreover, since this indicator also followed the same arterial dilution curve as Evan's Blue, $^{86}$Rb, or antipyrine it enabled the determination of the cardiac output at the same time and thus proved to be as useful as the previously employed $^{14}$C-antipyrine for the measurement of tissue perfusion [6]. These indicators may be used interchangeably to estimate flow in the brain up to rates of 1 ml/min/g although thiopental appears to be reliable up to rates of 1.5 ml/min/g (unpublished observations). Flow rates above these levels are underestimated because of diffusion limitations.

## RESULTS

*Estrogen.* In general, blood flows to most brain areas in the saline-injected diestrous female rats (Table 1) resembled those of litter-mate male rats and previously reported male rats [5] with the unique exception of a significantly lower perfusion rate for frontal cortex in the female. This difference, however, disappeared 10 min after acute estradiol administration as the flow to frontal cortex rose 44% (Table 2). Estradiol treatment in the female also elevated the flow of blood to most areas of the brain, significantly to the hippocampus, basal ganglia, and cerebellum 36, 33 and 27%, respectively (Table 2). All of these changes in flow occured in the absence of any modification of the cardiac output.

In the male, estradiol administration likewise elevated blood flow to most regions of the brain although the changes were smaller, averaging 8% (Table 3). Perfusion of the parietal cortex and, as in the female, cerebellum were most affected. Again these changes occurred without an altered cardiac output.

It should be noted that within five minutes after estrogen administration to both males and females even the most agitated animals reduced their activity, and evidence of fear such as excessive grooming and defecation was reduced substantially. The seemingly calmed animals appeared much more attentive to activities occurring outside their cage.

*MSH.* The outward behavior of animals 5–10 min after administration of αMSH and of estrogen were often alike particularly in their attentiveness to nearby events [5].

TABLE 1

REGIONAL BRAIN BLOOD FLOW IN ADULT, LITTERMATE MALE AND FEMALE RATS. NO ESTROGEN OR αMSH

| Tissue | Males | Females |
|---|---|---|
| pons & medulla | 0.85 ± 0.04 | 0.79 ± 0.08 |
| cerebellum | 0.89 ± 0.04 | 0.82 ± 0.08 |
| hypothalamus | 0.83 ± 0.04 | 0.78 ± 0.06 |
| basal ganglia | 0.89 ± 0.04 | 0.78 ± 0.08 |
| midbrain | 0.91 ± 0.04 | 0.83 ± 0.08 |
| hippocampus | 0.74 ± 0.04 | 0.67 ± 0.05 |
| olfactory bulbs | 0.69 ± 0.03 | 0.68 ± 0.05 |
| cortex | | |
| frontal | 0.99 ± 0.06 | 0.77 ± 0.06* |
| post-frontal | 1.04 ± 0.06 | 1.05 ± 0.11 |
| occipital | 1.06 ± 0.06 | 1.00 ± 0.06 |
| cardiac output | | |
| ml/min/kg | 333 ± 18 | 363 ± 10 |
| number of animals | 12 | 6 |

Blood flows are expressed in ml/min/g as means ± SE.
*$p<0.05$.

TABLE 2

REGIONAL BRAIN BLOOD FLOW IN ADULT *FEMALE* RATS 10 MIN AFTER INTRAVENOUS INJECTION OF ESTRADIOL-17β, 10 μg

| Tissue | Diluent only | Estrogen |
|---|---|---|
| pons & medulla | 0.79 ± 0.08 | 0.97 ± 0.06 |
| cerebellum | 0.82 ± 0.08 | 1.04 ± 0.06* |
| hypothalamus | 0.78 ± 0.06 | 0.96 ± 0.07 |
| basal ganglia | 0.78 ± 0.08 | 1.04 ± 0.07* |
| midbrain | 0.83 ± 0.08 | 1.03 ± 0.06 |
| hippocampus | 0.67 ± 0.05 | 0.91 ± 0.08* |
| olfactory bulbs | 0.68 ± 0.05 | 0.82 ± 0.05 |
| cortex | | |
| frontal | 0.77 ± 0.09 | 1.13 ± 0.08† |
| post-frontal | 1.05 ± 0.11 | 1.27 ± 0.09 |
| occipital | 1.00 ± 0.06 | 1.19 ± 0.09 |
| cardiac output | | |
| ml/min/kg | 363 ± 10 | 322 ± 23 |
| number of animals | 6 | 10 |

Blood flows are expressed in ml/min/g as means ± SE.
*$p<0.05$; †$p<0.02$.

However, those animals treated with MSH in infancy did not exhibit this kind of behavior at the time of the experiment, rather resembling the untreated animals. Unlike previously reported findings (Table 4), there were no significant differences in the perfusion of any part of the brain obtained from rats injected as infants with αMSH as compared to those injected with diluent (Table 5). Neither the group receiving MSH nor the group receiving the diluent differed significantly from saline-injected rats used in previous experiments [5].

Blood pH and gases were not significantly different from diluent injected rats for all groups, i.e., pH, 7.42 ± 0.01; $P_{O_2}$, 85 ± 2 mm Hg; $P_{CO_2}$, 41 ± 1 mm Hg.

TABLE 3

REGIONAL BRAIN BLOOD FLOW IN ADULT *MALE* RATS 10 MIN AFTER INTRAVENOUS INJECTION OF ESTRADIOL-17β, 10 μg

| Tissue | Diluent only | Estrogen |
|---|---|---|
| pons & medulla | 0.79 ± 0.02 | 0.86 ± 0.03† |
| cerebellum | 0.88 ± 0.02 | 0.98 ± 0.03† |
| hypothalamus | 0.84 ± 0.02 | 0.93 ± 0.03‡ |
| basal ganglia | 0.85 ± 0.02 | 0.92 ± 0.03 |
| midbrain | 0.89 ± 0.02 | 0.98 ± 0.03‡ |
| hippocampus | 0.74 ± 0.02 | 0.80 ± 0.03* |
| olfactory bulbs | 0.76 ± 0.02 | 0.82 ± 0.03 |
| cortex | | |
| frontal | 0.98 ± 0.03 | 1.07 ± 0.05* |
| parietal | 1.01 ± 0.03 | 1.14 ± 0.04† |
| occipital | 0.99 ± 0.03 | 1.08 ± 0.03* |
| cardiac output | | |
| ml/min/kg | 367 ± 15 | 382 ± 16 |
| number of animals | 22 | 12 |

Blood flows are expressed in ml/min/g as mean ± SE.
*$p<0.05$; †$p<0.025$; ‡$p<0.01$.

TABLE 4

REGIONAL BRAIN BLOOD FLOW 20 MIN AFTER ADMINISTRATION OF αMSH—40 μg/kg—DETERMINATION IN A PREVIOUS STUDY [5]

| Tissue | Diluent only | MSH |
|---|---|---|
| pons & medulla | 0.79 ± 0.02 | 0.72 ± 0.01† |
| cerebellum | 0.88 ± 0.02 | 0.81 ± 0.02† |
| hypothalamus | 0.83 ± 0.02 | 0.78 ± 0.02 |
| basal ganglia | 0.84 ± 0.02 | 0.79 ± 0.02 |
| midbrain | 0.89 ± 0.02 | 0.83 ± 0.02* |
| hippocampus | 0.74 ± 0.02 | 0.65 ± 0.02§ |
| olfactory bulbs | 0.75 ± 0.02 | 0.69 ± 0.02 |
| septum | 0.80 ± 0.02 | 0.74 ± 0.02 |
| cortex | | |
| frontal | 0.97 ± 0.02 | 0.90 ± 0.03 |
| parietal | 1.00 ± 0.02 | 0.91 ± 0.02‡ |
| occipital | 1.02 ± 0.02 | 1.03 ± 0.02 |
| cardiac output | | |
| ml/min/kg | 362 ± 11 | 314 ± 12 |
| number of animals | 32 | 13 |

Blood flows are expressed in ml/min/g as means ± SE.
*$p<0.05$; †$p<0.025$; ‡$p<0.01$; §$p<0.005$.

## DISCUSSION

Relatively small concentrations of estradiol and αMSH altered gross observable behavior very little soon after administration yet rapidly affected regional perfusion of the brains of conscious animals. These changes occurred in unique patterns quite unlike each other or those seen with more obvious behavior-modifying drugs such as LSD [9], psilocybin (unpublished observations), $\Delta^9$-tetrahydrocannabinol [8], ethanol [7], or pentobarbital [6]. As reported earlier [5] the flow of blood to most regions of the brain was reduced within 10 min after administration of MSH; only the occipital cortex was spared. Though these

TABLE 5

REGIONAL BRAIN BLOOD FLOW IN THE ADULT RAT PRETREATED WITH α MSH IN INFANCY

| Tissue | Diluent only | MSH |
|---|---|---|
| pons & medulla | 0.67 ± 0.05 | 0.66 ± 0.04 |
| cerebellum | 0.76 ± 0.06 | 0.79 ± 0.05 |
| hypothalamus | 0.75 ± 0.06 | 0.76 ± 0.04 |
| basal ganglia | 0.77 ± 0.05 | 0.77 ± 0.05 |
| midbrain | 0.78 ± 0.07 | 0.82 ± 0.05 |
| hippocampus | 0.65 ± 0.04 | 0.67 ± 0.06 |
| olfactory bulbs | 0.68 ± 0.03 | 0.73 ± 0.05 |
| septum | 0.70 ± 0.05 | 0.75 ± 0.07 |
| cortex | | |
| frontal | 0.93 ± 0.05 | 0.94 ± 0.06 |
| parietal | 0.91 ± 0.06 | 0.92 ± 0.06 |
| occipital | 0.98 ± 0.05 | 1.05 ± 0.06 |
| cardiac output | | |
| ml/min/kg | 338 ± 23 | 347 ± 15 |
| number of animals | 4 | 6 |

Blood flows are expressed in ml/min/g as means ± SE.

effects were transitory for most areas of the brain, perfusion of pons and medulla, cerebellum, hippocampus, and parietal cortex was still low after 20 min. By contrast, estradiol increased the flow of blood to most regions of the brain and to a greater extent in female than in male rats. We have assumed that the early regional flow responses to MSH and the regional uptake of this peptide [13] reflect possible functional changes in the brain which explain some of the effects of this hormone on learning and the subsequent behavior reported by several laboratories [2,27]. However, the ways in which estrogens affect behavior is still poorly understood despite considerable research [36].

Estrogens alter the functions of nervous tissues in ways which affect both tropic hormone secretion by the pituitary gland and the expression of specific patterns of behavior [36]. Although these steroids appear to have access to neurons generally [19,21], some regions of the brain appear to be especially sensitive. Labeled estrogens, for example, are tightly retained by neurons in the hypothalamus and preoptic areas [20,35], regions in which hormone implantation provokes characteristic patterns of sexual behavior. Furthermore, relatively nonspecific uptake data [23] as well as electrophysiologic [1, 14, 37], metabolic [10, 15, 22], and behavioral [18,24] evidence suggest that other regions including the olfactory bulbs and various parts of the limbic system are also target areas for estrogens.

With the notable exception of studies by Sawyer and his associates, however, emphasis in recent years has been mainly on the anatomy and dynamics of gonadal neuroendocrine control loops. "Yet the areas of the brain which are affected by steroids are so extensive as to suggest that the whole nervous system is influenced primarily, and localized systems of integrated behavior secondarily [14]". Our data support this concept and emphasize that the areas which respond to estrogen include, in addition to traditional endocrine target areas, many regions of the brains of both female and male rats. Additionally, the unique perfusion pattern and responsiveness of the female frontal cortex indicates a physiologic difference between male and female brains.

If it is assumed that regional blood flow in the brain in secondary to function, then the most parsimonious explanation for the findings with estradiol is that the acute flow changes signal parallel changes in the activities of several specific regions. Thus the unique patterns of perfusion in both female and male rats suggest possible anatomical substrates of behavior, such as frontal cortex, hippocampus, basal ganglia and cerebellum, which are especially responsive to estrogen and, as for MSH, provide a possible physiologic basis for future psychologic study.

In the case of αMSH, where appropriate behavioral indices have been studied recently, acute blood flow responses were followed by long-lasting changes in attentive behavior [29] and/or inhibition of extinction [2] lasting days or even months. Furthermore, behavioral effects were evident in adult rats who were injected with αMSH only in the first week of life [28]. These effects included increased aquisition of a difficult appetitive operant response, as well as, facilitated aquisition and reversal of a visual discrimination task, and were similar to previous findings with animals who were treated with MSH only as adults [30]. As Table 5 shows, however, the blood flow changes did not persist. Perfusion patterns in adult animals treated in infancy with MSH or an inert diluent were similar. The persistence of behavioral changes long after administration of MSH, therefore, does not necessarily require the presence of altered blood flow of the magnitude seen immediately after injection.

A substantial body of evidence supports the concept that the flow of blood within different parts of the nervous system is regulated by the functional neuronal activity within those parts [3, 12, 17, 26, 32, 33, 34]. Recently, for example, quantitative regional blood flow measurements have been used to estimate regional brain functions in cats during REM sleep [25] and in human beings during mental exercises [11]. It is not unreasonable, therefore, to expect that the effects of hormones, or of other drugs, on behavior are likewise accompanied by functional changes and secondarily by flow changes in specific responding areas of the brain. Our studies neither prove nor disprove this point. Although it seems more likely that a tissue change induced by MSH (and perhaps steroids) would persist from infancy to adulthood than would a change in blood flow, our results do not rule out the unlikely possibility that the initial tissue change was secondary to altered blood flow. More direct measures of metabolism are desirable on this point.

In summary, the acute flow alterations provoked by estradiol and αMSH, even in our small dissected samples of tissue, more than likely represented summations of heterogeneous microflows. For a net change in flow to be detected by our method at all suggests that most, if not all, of the tissue sample responded in the same functional and metabolic direction. That the flow changes exceeded levels reached by more obvious modifiers of behavior such as LSD, Δ⁹-tetrahydrocannabinol or alcohol, strongly suggest that the local metabolic and functional responses must have been substantial even though gross, overt behavior was less obvious. Such large yet transient responses may reflect adaptive processes in the brain which have consequences to future behavior that are profound.

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