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Cerebral Blood Flow After Treatment With ORG-2766, a Potent Analog of ACTH 4-9

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GOLDMAN, H., S. MURPHY, D. R. SCHNEIDER AND B. T. FELT. *Cerebral blood flow after treatment with ORG-2766, a potent analog of ACTH 4-9* PHARMAC. BIOCHEM. BEHAV. 10(6) 883–887, 1979.—Regional cerebral blood flows (rCBF) were measured in conscious, male rats at 10, 30, 60 min and 24 hr after intravenous administration of a potent, behaviorally active analog of ACTH/MSH 4-9 (ORG-2766). Flows in the basal ganglia, hippocampus, septal area and frontal cortex were depressed significantly throughout the 60 min postinjection period. Hypothalamic and parietal flows were depressed at 10 and 30 min, but recovered by 60 min, whereas flow to the cerebellum was depressed between 30 and 60 min postinjection. The least changed and therefore relatively better perfused area throughout the first 60 min period was the occipital cortex. By contrast, at 24 hr, when perfusion of all brain regions had returned to near control levels, flow to the occipital cortex was elevated. During the first hour after treatment with either ORG-2766 or αMSH the patterns of regional circulation in the brain were qualitatively the same. The data suggest that ORG-2766 and, probably, αMSH trigger serially linked neurophysiologic changes in the brain lasting at least 24 hr, which organize the behavioral actions of this class of peptides on memory and attentional processes.

Peptides ACTH αMSH Regional brain blood flow Occipital cortex Basal ganglia Hippocampus Septal area

POLYPEPTIDE fragments of ACTH/MSH profoundly affect memory and attentional processes [4,18]. The minimum peptide sequence capable of fully producing such behavioral changes is ACTH/MSH 4-7 [4]. Recently, a potent analog of ACTH 4-9, ORG-2766 (H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH), has been found to exert similar effects on behavior [2, 14, 23, 24, 33]. We have shown previously that αMSH quickly affected perfusion and, presumably, metabolic functions in most regions of the brains of conscious male rats: only the occipital cortex was spared [12]. The reduction of flow in the cerebellum, pons and medulla, hippocampus and parietal cortex persisted for at least 20 min after an intravenous injection.

Among the many questions raised by these observations was whether other polypeptides causing the same behavioral effects elicited the same blood flow pattern in the brain, i.e., was the flow redistribution uniquely related to both the peptide structure and the behavior.

We report here that the cerebrovascular effects of ORG-2766 and αMSH are similar and furthermore that some of these effects persist for at least 24 hr after administration.

METHOD

The blood flow method is a refinement of Sapirstein's indicator-fractionation technique [8]. Its utility relies on the fractional distribution of a single bolus of indicator, here ^{14}C-antipyrine. The method depends on the fact that the amount of the injected bolus of indicator taken up by a tissue is proportional to its exchangeable blood flow, as long as the tissue has the same extraction ratio towards the indicator as the whole body for some finite time after intravenous injection—here 20 to 60 sec. The validity of the method rests upon the demonstration that tissue uptake of the indicator does not change during this time, in spite of continuing recirculation. Under these conditions the ratio between tissue and whole body uptake of indicator equals the ratio between organ blood flow and cardiac output, i.e., the flow-fraction. Our modified method permits the estimation not only of the fractional distribution of the cardiac output but of the cardiac output as well, and thereby the minimum flow of blood which exchanges nutrients with a region.

Animals

Since the purpose of these experiments was to compare physiologic responses to ORG-2766 with those observed after administration of αMSH, conditions of the previous experiments were duplicated as closely as possible [12]. As noted above, the same indicator of flow was employed, despite its limitations (see Discussion), as were other conditions including strain and age of animal, experimental locale,

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TABLE 1

REGIONAL BRAIN BLOOD FLOW IN ADULT *MALE* RATS AFTER INTRAVENOUS INJECTION OF AN ACTH 4-9 ANALOG - ORG-2766

Tissue	Vehicle only	10 min	30 min	60 min	24 hrs
Pons and medulla	0.77 ± 0.01	0 73 ± 0.04	0 70 ± 0.02¶	0.73 ± 0.02	0.76 ± 0.02
Cerebellum	0.86 ± 0 01	0 79 ± 0 04	0 74 ± 0.03§	0.77 ± 0.02¶	0 83 ± 0.03
Hypothalamus	0.81 ± 0.01	0.71 ± 0.04¶	0.73 ± 0.02¶	0.78 ± 0.02	0.83 ± 0 03
Basal ganglia	0 82 ± 0 01	0.74 ± 0.04*	0.73 ± 0.01¶	0.75 ± 0 03*	0 83 ± 0 03
Midbrain	0 87 ± 0.01	0.82 ± 0.04	0.77 ± 0 03§	0.81 ± 0 02*	0 88 ± 0 03
Hippocampus	0.71 ± 0 01	0.60 ± 0 03§	0 64 ± 0.02†	0 64 ± 0.02§	0 71 ± 0 02
Septal area	0.76 ± 0 02	0 68 ± 0 04*	0 67 ± 0 02¶	0.69 ± 0.01§	0 81 ± 0 03
Olfactory bulb	0.72 ± 0 01	0 66 ± 0 03	0 61 ± 0.01§	0 67 ± 0.01*	0 74 ± 0 02
Cortex					
occipital	1.05 ± 0.02	1 02 ± 0.05	1 00 ± 0.02	1.03 ± 0 03	1.16 ± 0.03†
parietal	0 99 ± 0.02	0.87 ± 0.04†	0 88 ± 0 02¶	0 92 ± 0 02	1 00 ± 0 03
frontal	0.94 ± 0 02	0 84 ± 0.04†	0.81 ± 0.03§	0.86 ± 0 02¶	0.94 ± 0 03
Cardiac output					
ml/min/kg	372 ± 9	369 ± 23	322 ± 11*	339 ± 11	412 ± 15
Arterial blood					
pH	7 42 ± 0.00	7 44 ± 0.01	7.42 ± 0.01	7 42 ± 0 01	7 41 ± 0 01
$PaCO_2$	40 ± 1	34 ± 1§	39 ± 1	37 ± 1*	40 ± 1
PaO_2	86 ± 1	90 ± 1	86 ± 2	85 ± 2	81 ± 4
Animals	48	8	10	12	12

Blood flows are expressed in ml/min/g as means ± SE. *$p<0.025$, †$p<0.01$; ¶$p<0.005$; §$p<0$ 001, compared to vehicle control.

lighting, sound level, apparatus, time of day and season, and laboratory personnel.

Since in our previous studies of αMSH [12] perfusion of the occipital cortex was uniquely affected, particular attention was paid to duplicating lighting levels and schedules, as well as informational input. Lighting schedules were maintained 0700 hr on, 1900 hr off. All experiments were performed between 0900 and 1200 hrs. During the experiment, as noted below, all animals were kept in the same black plastic boxes which contained 1/4 in. slit windows. Ambient light levels at these windows were maintained between 340 and 440 LUX. All experiments were performed in the same room and orientation, as well as by the same personnel.

These studies were performed in male Wistar rats, 75–85 days of age. All animals were line-bred by us, and maintained under strict environmental regulations including controlled maternal and neonatal nutrition. Each treatment group listed in Table 1 consists of representatives from no less than five litters.

Procedure

Three days before flow was measured, 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. At 10, 30, 60 min and 24 hr prior to measurement of flows, ORG-2766 was injected through the venous catheter: each animal received 40 μg/kg of the hormone (≈17 nanomoles/rat), a behaviorally effective dose, in an approximate volume of 0.1 ml of vehicle consisting of 0.01 N acetic acid:0.85% saline. Control animals received the equivalent volume of vehicle only or saline.

On the day of the measurement, each animal was placed in a small black plastic box at t=−10 min, the catheters were freed from the neck cap. The 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=−1 min, 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 (5 μCi of ^{14}C-antipyrine in 120 μl of saline) was flushed smoothly into the circulation through the femoral vein catheter 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 started for the determination of the cardiac output by an indicator-dilution technique [30] using the ^{14}C-antipyrine indicator Collection of arterial blood ended at t=15 sec, and at t=20 sec the animal was killed by a rapid, intravenous injection of 250 μl of a saturated KCl solution.

Subcortical regions were dissected according to the protocol of Glowinski and Iversen [7]: telencephalic areas, described as frontal, parietal and occipital, correspond to the parcellation of Krieg as areas 10, 1–3 and 17–18, respectively [19]. Tissue indicator was extracted (>98%) by the Bray's solution solvents and counted in a liquid scintillation spectrometer.

RESULTS

Since by statistical analysis the various control animals showed no significant regional variations in flow, all control values have been pooled in Table 1. It should be noted that regional cerebral blood flow (rCBF) values of these control animals were not significantly different from the many uninjected or saline injected control animals which we have examined in the past.

As in the case of αMSH [12], the flow of blood to all areas of the brain, except the occipital region, decreased soon after intravenous administration of the synthetic polypeptide, ORG-2766. However, the effects were more pronounced and prolonged. As seen in Table 1, decreases were significant in hypothalamus, basal ganglia, hippocampus, septal, parietal and frontal cortical areas within 10 min. By 30 min, flows to all regions except occipital cortex were significantly depressed by 9 to 15%. Although flows appeared to be returning to near control levels by 60 min, they were still depressed significantly in the cerebellum, basal ganglia, midbrain, hippocampus, septal area, olfactory bulb, and frontal cortex. During the first hour the sole unaffected area of the brain remained the occipital cortex.

At 24 hr post-injection, flows to all areas of the brain had recovered to control levels. By contrast, perfusion of the occipital cortex was elevated significantly by 12%.

A reduction of the cardiac output by about 10% between 30 and 60 min contributed to the lowered rCBF at these times (MANOVA analysis). At all other times, however, cardiac output was not different compared to vehicle-injected or previously reported saline treated control animals [8]. Blood pH and arterial partial pressures of oxygen (PaO_2) were constant throughout the experiment. However, at 10 min and 60 min, $PaCO_2$ was significantly lower by 15 and 8%, respectively (Table 1).

Other animals, observed only for gross behavior, became slightly dystonic between 5 and 15 min after drug treatment: when picked up they made few attempts to escape. A similar response was seen after injection of αMSH [12]. Although they moved little and were relaxed when held, these animals were not drowsy: rather, they were attentive to nearby events. Rapid breathing, which was constantly observed during this interval of 10 min, could explain the hypocarbia observed in the experimental animals (Table 1). However, such hyperventilation was not obvious at 60 min when hypocarbia again occurred.

Hypocarbia at 10 and 60 min postinjection probably was partially responsible for reduced circulation in the brain through an action on cerebral vascular smooth muscle (Table 1). However, regional flows were their lowest at 30 min under normocapnic conditions.

Tissue	Time			
	10 min.	30 min.	60 min.	24 hr.
pons & medulla	•	•	•	•
cerebellum	•	•	•	•
hypothalamus	↓	•	•	•
basal ganglia	•	•	•	•
midbrain	•	•	•	•
hippocampus	↓	•	•	•
septal area	•	↓	•	•
olfactory bulb	•	↓	•	•
cortex, occipital	•	•	•	↑
parietal	↓	•	•	•
frontal	•	↓	•	•

FIG. 1. Changes in rCBF following intravenous injection of ORG-2766—after adjustment for the effects of $PaCO_2$ and cardiac output covariates (MANOVA) Responses are significant at the $p<0.05$ level

DISCUSSION

The behavioral effects of ORG-2766 and αMSH are similar [33]. Both facilitate processes of memory retrieval [33] and attention [2, 14, 23, 24, 27, 28, 33], and both inhibit extinction of learned behavior patterns [25,26]. Qualitatively, the effects of ORG-2766 and αMSH [12] on regional cerebral circulation in rats likewise are similar. That is, the perfusion of most areas of the brains of conscious male rats is reduced soon after injection of these peptides: only the occipital cortex is spared.

Quantitatively, the effects of ORG-2766 are more intense and prolonged. This is expected in view of ORG-2766's greater behavioral potency [23] and longer half-life in the systemic circulation [37]. We assume that this cerebrovascular response is common to peptides with similar behavioral effects. By contrast, a variety of disparate drugs with other behavioral and neurophysiologic actions, such as pentobarbital [8], alcohol [9], LSD [11], Δ^9-THC [10], and psilocybin (unpublished observations), as well as steroid hormones such as estrogen [13], provoke altered patterns of cerebral perfusion which are distinctive and different.

Careful comparison of the data reveals that treatment with ORG-2766 also significantly affects the partial pressure of CO_2 ($PaCO_2$) in arterial blood at 10 min, and the cardiac output between 30 and 60 min postinjection. Both of these factors can influence significantly the perfusion of the brain, as well as other regions of the body. By the use of multivariate analysis (MANOVA), and with covariate compensation for the contribution of altered $PaCO_2$ and cardiac output to changes in rCBF, it can be shown: (1) that significant responses to treatment with ORG-2766 are still detectable in the hypothalamus, hippocampus, septal area, olfactory bulb, and the parietal, frontal and occipital cortex (Fig. 1): (2) that these regional responses vary with time: and (3) that the perfusion of the brain as a whole is affected ($p<0.003$) for as long as 24 hr after the single injection of ORG-2766, more than 300 half-lifetimes [35]. The consequences of these time dependent reductions in $PaCO_2$ and cardiac output on the circulation of regions other than brain are under investigation.

Accurate measurement of regional cerebral blood flow by our method requires that the indicator of flow is freely diffusible: its extraction must be relatively complete in a single pass through a tissue mass. Antipyrine, previously thought to meet this criterion, has been found to be diffusion limited [5, 6, 22] so that flows in several regions of the brain tend to be underestimated. Since the magnitude of the error is flow-dependent, the range of regional blood flow values tends to be compressed. Responses to conditions which alter flow therefore tend to be similarly distorted.

The blood flow responses to ORG-2766 in these experiments were determined under experimental conditions which were identical to those reported in a previous study of αMSH [12]. Although the observed circulatory responses to both of these substances therefore may be compared directly, they now must be considered semiquantitative due to the diffusion limitation of the antipyrine indicator. It is very likely, however, that the magnitudes of such changes are larger than can be inferred from the data in Table 1.

It might be argued that since the extraction of antipyrine is diffusion limited to some extent, its altered regional uptake may be due to shifts in capillary permeability rather than to changes in blood flow. Accordingly, the lower content of

antipyrine in several brain regions found soon after administration of ORG-2766 may have been due to reduced diffusibility of antipyrine. To what extent this might have occurred was determined in a separate group of animals drawn from the same colony using a modified version of the technique of Oldendorf [21]. With this method, the brain uptake of a freely diffusible substance such as butanol-^{14}C [22] was compared to that of antipyrine-^{3}H after their simultaneous intracarotid injection. During the first 30 min after injection of ORG-2766, when both behavior [33] and the uptake of antipyrine should have been most affected, the extraction of antipyrine relative to butanol was not reduced. In an unpaired *t*-test comparison of four placebo and seven drug treated rats, the probability of a lower extraction of antipyrine relative to that of butanol was $p<0.001$. We conclude, therefore, that the regional changes in the content of antipyrine observed after treatment with ORG-2766 reflect changes in blood flow.

Regional cerebral blood flow usually is considered to be regulated by local neuronal or glial function and metabolism. Thus, the diminished flows we have observed could be construed as reflecting decreased function in most of the brain regions examined: the consequences of unchanged flow in the occipital cortex might reflect a relative increase in visually related function. While this flow-function relationship seems to hold in many reported instances [17], it may not be entirely so here. It is noteworthy that although the blood flows to most regions of the brain, especially to the brain stem and cerebellum (Table 1), often were lower than those found in anesthetized animals in which function and metabolism are generally reduced [15,32], animals treated with a behaviorally effective dose of ORG-2766 were not only conscious but attentive to their surroundings. Our findings suggest, therefore, that other, equally prominent determinants of cerebral blood flow besides local metabolism may play a role here. This view is supported by recent observations that the metabolism of glucose in the brains of male mice, as estimated by the regional uptake of ^{3}H-2-deoxyglucose, appears to be unaffected by treatments with αMSH or behaviorally active analogs of ACTH [3].

How the mechanisms of action of ORG-2766 or αMSH relate to memory and attentional processes is still a mystery. Nevertheless, unique changes in both rCBF, as well as cyclic nucleotide levels [31] implicate certain areas of the brain which may underlie the unique behavioral responses to such peptides. In particular, we call attention to possible frontal cortical, hippocampal, septal, and striatal, as well as occipital cortical roles in such processes. This latter area, showing a unique flow response throughout a 24 hr period after injection, strongly implicates visual mechanisms in the attentional and memory processes that are modified by ACTH/MSH fragments. This supports behavioral studies in both rodents and humans [20, 26, 29]. However, the temporal patterns of rCBF, as well as of 3′,5′-cyclic adenosine monophosphate (cAMP) [31], suggest that a long-lasting series of physiologic and psychologic processes are triggered by ORG-2766, a peptide with a plasma half-life of about 5 to 15 min [35,37]. Thus, while changes in rCBF and attentional behavior [1,28] reach a maximum between 30 and 60 min post-injection in the rodent, the persistence of changes in flow to the occipital cortex and of cAMP content in other regions of the brain strongly suggest that other, more protracted behavioral effects are likely to be found which last at least 24 hr.

It should be emphasized that although our approach suggests anatomic areas which are unusually responsive to these hormone fragments, it is unlikely to discriminate between primary and secondary sites of action. For example, although changes in rCBF in the septal area, olfactory bulb and cerebellum correlate with the binding of ^{3}H-ORG-2766 in these areas [34,36], the changes in rCBF in the frontal cortex, hippocampus, basal ganglia and midbrain structures occur in the absence of detectable uptake of ORG-2766 [34,36]. It may be that functional and metabolic responses in these latter regions are secondary to responses of other regions of the brain.

However the data are interpreted, the effects of the potent, short-lived drug, ORG-2766, appear to be long-lasting and selective for certain regions of the brains of male rodents. These unique changes in regional circulation and cAMP content not only provide clues to possible mechanisms of action for this type of very potent modifier of behavior, but they also suggest cellular mechanisms which may underlie the processes of memory, attention and possibly other types of behavior.

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