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An Analog of ACTH/MSH$_{4-9}$, ORG-2766, Reduces Permeability of the Blood-Brain Barrier

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GOLDMAN, H. AND S. MURPHY. *An analog of ACTH/MSH$_{4-9}$, ORG-2766, reduces permeability of the blood-brain barrier.* PHARMAC. BIOCHEM. BEHAV. 14(6) 845–848, 1981.—Regional uptakes of a diffusion-limited substance, antipyrine, were compared to those of a highly diffusible substance, iodoantipyrine, in brains of conscious, unrestrained rats. The method included simultaneous measurements of regional cerebral blood flow. Within 10 min after intravenous injection of a behaviorally active analog of ACTH/MSH$_{4-9}$, ORG-2766, the relative extraction of antipyrine was reduced in most regions of the brain, significantly in hypothalamus, hippocampus, parietal cortex and frontal cortex. The occipital cortex and brain stem were least affected. Since the flow of blood was not changed significantly in any region at this time, we conclude that the changes in extraction reflect a reduction in permeability of the blood-brain barrier. These results suggest that the behavioral responses to peripherally administered fragments of ACTH/MSH may depend, in part, on some action in the blood-brain barrier. These observations also suggest a mechanism by which such peptides may influence the behavioral effects of diffusion-limited drugs.

ACTH/MSH Permeability Blood-brain barrier Regional cerebral blood flow

IN PREVIOUS studies of the cerebral circulatory responses to αMSH and a behaviorally potent analog of ACTH$_{4-9}$, ORG-2766, we described regional cerebral blood flow (rCBF) to be significantly reduced within minutes after the intravenous injections of the peptides [8, 9, 11]. The regional responses were qualitatively similar for both peptides; responses to the more potent drug predictably were greater. In these experiments, the regional uptake of antipyrine was believed to be related solely to the flow of blood since the diffusion of this indicator from the vascular space into the brain was not considered to be limiting [20].

Recently, antipyrine uptake in the brain has been shown to be diffusion-limited [4, 5, 24] and, therefore, can lead to underestimation of rCBF; the magnitude of the error is related to the rate of blood flow. Since rCBF tends to be high in the rat, errors can be large. Using a more reliable indicator of blood flow, iodoantipyrine [15,24], we find that our previous estimates of rCBF were in error and that the changes in regional uptake of antipyrine observed shortly after treatment with ORG-2766 are the result mainly of changes in extraction and not rCBF.

METHOD

The method and experimental protocol for the measurement of regional cerebral blood flow (rCBF) are the same as those used previously [9, 10, 11], except that iodoantipyrine, a lipid soluble indicator with few diffusion limitations [15,24], replaced antipyrine as the indicator of flow. In addition, the method was extended to include measurements of extractions of antipyrine in eleven regions of the brains of conscious, unrestrained, male rats by employing a modification of Oldendorf's technique [1,17]. This combination of methods is described below. Experiments were conducted in the same line-bred strain of Wistar derived rats used previously [8, 9, 11], at the same age, and under near identical conditions including experimental locale, lighting schedule, ambient sound level, apparatus, time of day and season, and laboratory personnel.

Measurements and Calculations

The study includes two groups of animals, treated either with placebo or drug, in which regional cerebral uptakes of antipyrine were determined. For partially diffusible substances, such as antipyrine, i.e., where the permeability coefficient is of the order of 10^{-4} cm sec^{-1}, blood flow must be taken into account to determine the extraction of the tracer during its passage through the brain. Therefore, the study employed an additional pair of groups of similarly treated animals in which the uptake of the readily diffusible marker, iodoantipyrine, served both as an index of regional cerebral blood flow, as well as a reference for antipyrine uptake from which regional extraction values were subsequently derived.

Ideally, in this modification of the Oldendorf method, the uptake of a diffusion-limited substance relative to a freely diffusible one is best determined in the same animal. Detec-

TABLE 1

REGIONAL CEREBRAL EXTRACTION AND PERMEABILITY OF ANTIPYRINE AND BLOOD FLOW AFTER INTRAVENOUS INJECTION OF AN $ACTH_{4-9}$ ANALOG, ORG 2766

Tissue	Blood Flow		Extraction		Permeability·Surface Area	
	Placebo	Drug	Placebo	Drug	Placebo	Drug
Pons and Medulla	1.05 ± 0.02	1.00 ± 0.04	0.74 ± 0.01	0.74 ± 0.03	243 ± 9	235 ± 21
Cerebellum	1.08 ± 0.02	1.11 ± 0.05	0.80 ± 0.01	0.73 ± 0.03	304 ± 14	257 ± 23
Hypothalamus	1.19 ± 0.02	1.21 ± 0.08	0.69 ± 0.01	0.62 ± 0.03‡	235 ± 7	199 ± 15*
Basal Ganglia	1.10 ± 0.02	1.06 ± 0.04	0.75 ± 0.01	0.72 ± 0.03	263 ± 11	233 ± 17
Midbrain	1.23 ± 0.02	1.26 ± 0.06	0.71 ± 0.01	0.66 ± 0.03	260 ± 8	234 ± 16
Hippocampus	0.88 ± 0.02	0.87 ± 0.04	0.81 ± 0.01	0.72 ± 0.03‡	245 ± 11	191 ± 14†
Septal Area	1.03 ± 0.02	1.00 ± 0.04	0.74 ± 0.02	0.71 ± 0.03	245 ± 14	215 ± 19
Olfactory Bulb	0.89 ± 0.02	0.86 ± 0.04	0.79 ± 0.01	0.78 ± 0.03	245 ± 11	226 ± 18
Cortex						
Occipital	1.66 ± 0.04	1.75 ± 0.09	0.63 ± 0.01	0.60 ± 0.02	284 ± 9	270 ± 16
Parietal	1.38 ± 0.02	1.43 ± 0.09	0.72 ± 0.01	0.64 ± 0.03‡	307 ± 12	251 ± 20*
Frontal	1.27 ± 0.02	1.29 ± 0.07	0.75 ± 0.01	0.67 ± 0.03‡	307 ± 13	242 ± 18*
Animals	14	9	47	11	47	11
Caridac Output ml/min/kg	322 ± 7	314 ± 9				
Arterial Blood pH	7.41 ± 0.00	7.42 ± 0.03				
$PaCO_2$	42 ± 1	39 ± 1				
PaO_2	79 ± 1	80 ± 2				

Blood flows are expressed in ml/min/g; extractions as ratios of antipyrine/iodoantipyrine relative specific activities; permeability coefficient·capillary surface area product in ml/sec/g$\times 10^{-4}$. All values are means ± SE.

*$p<0.05$; †$p<0.025$; ‡$p<0.01$.

tion of such pairs of substances in a tissue requires differential radiolabelling, usually with ^{14}C and ^{3}H. However, relatively stable forms of antipyrine and iodoantipyrine are available commercially only with a ^{14}C label; ^{131}I or ^{125}I labeled forms of iodoantipyrine proved to be unreliable indicators due to radiolytic breakdown.

The permeability of antipyrine was considered in terms of a model proposed by Renkin [21] and Crone [3] for the loss of diffusible substances from single capillaries. The model assumes that diffusion of material from a capillary is unidirectional, that the rate of diffusion is proportional to the remaining concentration at every point in a capillary, and that the transit time through each capillary in a region is the same. In this model, the loss of a diffusible substance, such as antipyrine, from a capillary is related to its permeability and the flow of blood according to the equation, $\ln(1-E) = -PS/F$, where P is the permeability coefficient, S is the surface area of the capillary, E is the extraction of the diffusible substance, and F is the blood flow. The values for the regional extractions of antipyrine (E_A), as presented in Table 1, were determined from the ratio of the relative specific activity of ^{14}C-antipyrine and the mean relative specific activity of ^{14}C-iodoantipyrine in corresponding regions of the brain. The relative regional specific activity of either indicator was taken as the ratio of the regional content of indicator and its respective integrated arterial concentration determined between the time of initial arterial delivery to the brain and 13 sec thereafter. The time-course of transport of both tracers [1] was assumed to be the same so that E_A remained constant during this 13 sec period of measurement.

Student's *t* test was used to compare blood flows, relative extraction of antipyrine, and PS products for eleven regions of the brains of rats 10 min after an intravenous injection of ORG-2766 or a placebo.

Experimental Procedure

Three days before regional uptake and blood flow measurements were performed, rats were lightly anesthetized with chloroform. PE-50 polyethylene catheters were placed in one femoral vein and the opposite femoral artery. The catheters were filled with a heparin solution and heat sealed. Both 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.

On the day of the measurement, each animal was placed in a black plastic box at t=−11 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. Ten minutes prior to measurement, ORG-2766 was injected through the venous catheter: each animal received 40 μg/kg of the peptide, about 17 nanomoles/rat, in a volume of 0.1 ml of vehicle containing 0.01 N acetic acid: 0.85% saline. Control animals received the equivalent volume of vehicle only or saline. 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 or ^{14}C-iodoantipyrine in 120 μl of saline) was flushed smoothly into the circulation through the femoral vein catheter over a one sec period. Also at t=0, collection of one sec samples of arterial blood, about 15 μl

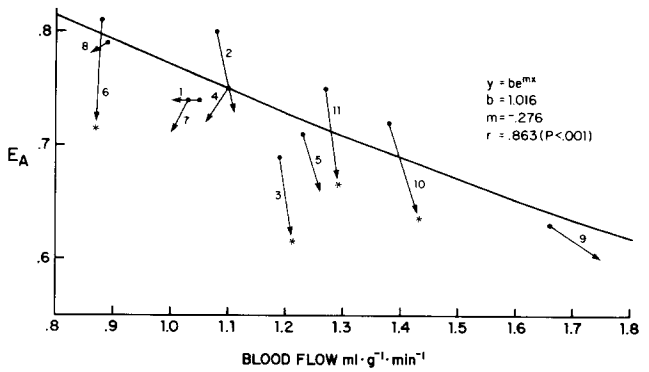

FIG. 1. Illustration of inverse correlation of mean regional extraction of antipyrine (E_A) with regional cerebral blood flow and shifts in extraction 10 min after treatment with the peptide, ORG-2766. Brain regions are numbered as follows: 1—pons and medulla, 2—cerebellum, 3—hypothalamus, 4—basal ganglia, 5—midbrain, 6—hippocampus, 7—septal area, 8—olfactory bulb, 9—occipital cortex, 10—parietal cortex, 11—frontal cortex. Treatments: ○ placebo; → drug; $*p<0.01$.

each, was started for the determination of the cardiac output by an indicator-dilution technique [26] using the ^{14}C-iodoantipyrine indicator. Collection of arterial blood samples ended at t=15 sec after 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 [14]. Tissue indicator was extracted (>99%) by the Bray's solution solvents and counted in a liquid scintillation spectrometer. In these experiments, mean cerebral transit time of the injected bolus of indicator was estimated to be 3.5 sec; death occurred 8.5 sec after the mean peak cerebral content was reached.

RESULTS

Regional cerebral blood flow (rCBF), determined more accurately by means of the iodoantipyrine indicator, are shown in Table 1. Values for rCBF agree with those determined by a different method [15,24]. The uptake of antipyrine compared to iodoantipyrine was lower in every region and correlated well ($p<0.001$) with the reciprocal of the regional blood flow (Fig. 1).

Treatment with ORG-2766 did *not* affect blood flow significantly in any of the regions under study, although there was a detectable increase in half of them. However, within 10 min after intravenous injection of the peptide, the uptake of antipyrine was reduced in every region of the brain except occipital cortex and brain stem; extraction (E_A) of this indicator relative to that of iodoantipyrine was reduced significantly in the hypothalamus, hippocampus, parietal cortex and frontal cortex. The permeability—capillary surface area products (PS), calculated from the regional values of flow and extraction, likewise were reduced significantly in these regions. By contrast, E_A and PS appeared to be least affected in the occipital cortex and brain stem after treatment with ORG-2766.

DISCUSSION

The uptake of antipyrine, when compared to that of iodoantipyrine, was lower in every region of the brains of all animals. This was an expected consequence of the limited diffusibility of antipyrine [4, 15, 24]. In control animals, this relative extraction of antipyrine (E_A) varied regionally from 63 to 87%. Much of the regional heterogeneity appeared to depend on the differences in regional cerebral blood flow (rCBF) and the inverse relationship which exists between extraction and flow ([3,21], Fig. 1). The diffusion limitation of antipyrine has been exploited here to demonstrate and quantify regional differences in the responses of the blood-brain barrier to the behaviorally active analog of ACTH/MSH_{4-9}, ORG-2766.

In control animals, the high negative correlation between regional extraction and rCBF (Fig. 1) suggests that the permeability coefficient—capillary surface area products (PS) for antipyrine were fairly uniform in the brain, although they tended to be somewhat higher in cerebellum and cortical areas than in subcortical areas. This may have been due either to true differences in regional permeability or more likely to variations in capillary surface areas associated with differing mixtures of grey and white matter [19]. In brains of conscious, unrestrained control animals, the average of the regional PS values for antipyrine was 0.026 $cm^3 \cdot sec^{-1} \cdot g^{-1}$. Variations between regions were small, SD<11%. This compares favorably with Eckman's PS determination for antipyrine in whole brain, 0.02 $cm^3 \cdot sec^{-1} \cdot g^{-1}$ [4].

In contrast to our previous report [8], treatment with ORG-2766 did not reduce rCBF significantly in any region of the brain when flow was determined by means of iodoantipyrine, an indicator with reportedly few diffusion limitations [4, 15, 24]. However, it is clear that the regional extraction of antipyrine was reduced quickly and non-uniformly in the brain. Thus, changes in uptake of antipyrine, equated in the past with rCBF, could be explained in every case by reduced extraction from arterial blood as it traversed a local tissue mass. Within 10 min after intravenous injection of the peptide, regional E_A ranged from 59 to 77%, a significant drop from levels observed in control animals. However, E_A was least affected in occipital cortex and brain stem, whereas it was reduced significantly ($p<0.01$) in hypothalamus, hippocampus, parietal cortex and frontal cortex (Fig. 1). Since rCBF was relatively unaffected by treatment with the peptide, regional PS products for antipyrine paralleled changes in E_A (Table 1). Assuming that capillary surface area remained constant with a given region [3, 15, 19], the differential uptake of antipyrine in the four functionally interrelated areas of the brain, hypothalamus, hippocampus, parietal and frontal cortex, probably reflected altered regional permeabilities.

In view of these findings and of previously reported variations in the regional uptake of antipyrine over a 24 hour period following injection of ORG-2766 [8], it is likely that extraction of antipyrine is most affected in the rat brain at 30 min and returns to predrug levels between 1 and 24 hours. Our estimates for average whole brain extraction of antipyrine is 74% in placebo treated animals; 69%, 66%, 68% and 75% at 10 min, 30 min, 60 min and 24 hours after drug treatment, respectively. Viewed in another way, the peak effect on cerebrovascular permeability probably occurs within two half-lives of ORG-2766 in plasma ($t_{1/2} \simeq 16$ min, personal communication, H. van Riezen, Organon) and probably is without significant effect beyond ten half-lives. This suggests that the actions of the peptide on cerebrovascular permeability are reversible and that they closely follow the time-course of effectiveness of ORG-2766 on behavioral

indices [6, 22, 25, 28, 29] after peripheral administration.

The capillaries of the brain are structurally and functionally constituted to regulate the passage of solutes between the blood and the brain. Intercellular junctions which join the endothelial cells of such capillaries form an effective barrier in most regions of the brain so that solutes are obliged to pass through a series of limiting physical and chemical interfaces in these cells in order to reach the brain [18]. In the absence of facilitated transport mechanisms, the passive movement of antipyrine, as well as most other drugs, through endothelial cells depends mainly on the molecule's affinities for water and membrane lipid as well as the nature of the cellular surfaces which must be traversed [16]. We speculate that ACTH/MSH and some of their fragments are able to alter passive transport mechanisms in cerebrovascular endothelium and thereby regulate the entry of antipyrine and, most likely, other diffusion-limited substances into the brain. The consequences of this mechanism on the behavioral effects of a variety of drugs could be significant.

In conclusion, it is generally agreed that the behavioral effects of ACTH/MSH peptide and their fragments can arise from peripheral injection [13]. Nevertheless, remarkably small amounts of these peptides are reported to reach the brain from the systemic circulation [2, 12, 13, 27]. Recently, melanotropic peptides have been found to alter blood-CSF barriers [23]. Our own observations extend this idea and raise the possibility of a reversible, permissive role for these peptides in the regulation of transport processes located within the blood-brain barriers, as well. This may help explain some of the actions of ACTH/MSH hormones on behavior and their interactions with other behavior modifying drugs.

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