EXPERIMENTAL NEUROLOGY 31, 200–206 (1971)

Stimulus Deprivation and Cerebral Blood Flow

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Received January 6, 1971

The effect of unilateral visual deprivation on the vascular system of brain regions has been studied in the chick where the visual pathways are totally crossed. We injected ^{125}I-iodantipyrine or ^{125}I-iodinated albumin into the hearts of chicks at various times after monocular eyelid suture or unilateral eye extirpation. Radioactivity within optic lobes and cerebral hemispheres was assayed, to estimate the velocity of blood flow or the plasma volume. As early as 1 hr after monocular deprivation by eyelid suture or by enucleation, significant deficits in the velocity of the circulation through the contralateral brain regions were observed. These effects were maintained for at least 6 days and were of similar magnitude in contralateral optic lobes (which are directly innervated by the optic nerve) and the contralateral cerebral hemispheres (which receive no primary innervation from the optic nerve). After 1 hr of either form of monocular deprivation, a reduced plasma volume was found in contralateral optic lobes but not in cerebral hemispheres. These data suggest that maintenance of optimal cerebral vascular supply may be dependent on a patterned sensory input to the brain. A deficiency in the complexity of cerebral afferentation in the young animal may impair adequate vascularity and thus retard maturation.

Introduction

Many physiological factors govern the adequate development of the central nervous system. Those critical factors which limit this process may determine the level of cerebral functioning achieved. The high requirement of cerebral tissue for glucose and oxygen make it likely that one such limiting parameter may be the rate at which nutrients are supplied to the brain by the vascular system.

This study reports the effects of various forms of visual deprivation on cerebral blood flow. An experimental model was employed utilizing chicks that had been monocularly visually deprived by unilateral eye enucleation or by eyelid suture. These procedures have been described (2). In brief, the advantages of such a system are: (a) The complete decussation of the

[1] This work was supported in part by grant No. 70-487 from the Foundations' Fund for Research in Psychiatry. Mrs. Morelos' present address is Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, California, 90024.

avian optic tract, in conjunction with the absence of major interhemispheric commissures such as a corpus callosum reduces interactions between the two symmetrical halves of the brain (8). (b) Consequently, the metabolic deficits caused by unilateral visual deprivation are largely confined to regions contralateral to the treated eye (3, 10). (c) The avian brain is symmetrical with no left or right dominance being observable (7). (d) Regions that are primarily or secondarily innervated by the treated eye can be compared to the corresponding unimpaired region in the same animal. Thus, any difference observed between paired experimental and control regions cannot be attributed to systematic, hormonal variations which should affect both regions equally and must thus be directly effected by the experimental procedure. (e) By the use of paired regions within a single animal rather than paired individual animals, small differences can readily be distinguished.

Methods

Fertile eggs from white Leghorn strain K137 were incubated at 37.5 C until hatched. The newly hatched chicks were maintained in a brooder. Within 24 hr of matching, the chicks were lightly anesthetized with halothane and a single eye was removed or a single pair of eyelids were sewn shut. Left and right eyes were alternately treated. Animals recovered from anesthesia within 10 min. At various times after this procedure, the velocity of blood flow and the total plasma volume within cerebal hemispheres and optic lobes was determined.

Blood Flow. After various periods of unilateral visual deprivation, chicks were injected intracardiacly with 0.15 ml saline (0.14M) containing 3 μc of 4-(^{125}I)-iodantipyrine (120–200 μc/m-mole) or 2 μc of 2-(^{14}C)-barbituric acid (2.0 mc/m-mole). A 19 mm number 27 gauge needle was used. Ten seconds after this injection, the birds were decapitated and individual cerebral hemispheres and optic lobes dissected out on ice. These regions were then rapidly weighed and placed in glass scintillation vials. One milliliter of NCS solubilizer (Nuclear-Chicago Corp.) was added to each vial and the tissues dissolved in 1 hour at 70 C. After cooling, 10 ml of scintillation solution was then mixed into each sample. This consisted of toluene containing 0.5% 2-phenyl-5-(biphenyl-2-yl)-1,3,4, oxidiazole and 0.01% 1-4 bis (5-phenyloxazole-2-yl) benzene. Radioactivity was measured in a Packard Tri-Carb scintillation spectrometer under appropriate conditions. Quench corrections were made, when needed, by the use of an external standard. Data were calculated as counts/min/mg tissue for each sample.

Plasma Volume. Radioiodinated albumin was prepared from bovine serum albumin as follows. Two or three milligrams of protein were dissolved in 2 ml of sodium phosphate buffer (0.05M, pH 7.4) and 10 μl of

a carrier free K ^{125}I solution containing 1 mc was added. Then 0.5 ml of a freshly prepared solution of Chloramine T (200 μg/ml) was stirred together with this in ice for 5 min. Reaction was then stopped by the addition of 0.5 ml sodium metabisulfite (200 μg/ml). The mixture was then passed through a Sephadex column (G25, 1 $\times$ 45 mesh) previously equilibrated with isotonic saline. Labeled albumin was eluted from this column with isotonic saline solution, as a discrete peak of absorbance at 280 mμ, which coincided with the radioactive peak.

Subsequently, 0.2 ml of this fraction (containing 1.1–3.8 $\times$ 10^6 counts/min) was intracardiacly injected into chicks, after 1 hr of visual deprivation. After allowing 1.5 min for the albumin to reach circulatory equilibrium, chicks were killed by immersion in liquid nitrogen at $-$ 198 C. After allowing carcasses to warm to around -5 C, semifrozen brains were removed and cerebral regions dissected out. They were immediately placed in preweighed vials which were then weighed again. Regions were then dissolved in 1 ml NCS solubilizer and assayed for radioactivity as described above.

Statistics. Each experimental point was repeated on 7–28 chicks and radioactivity was never less than 600 counts/min for any cerebral region assayed. Probability of these internally paired sets of data was calculated by a Student's one-tailed t test with $p<0.05$ taken to be significant.

Regional Blood Flow. The isotope chiefly used for injection was 4-(^{125}I)-iodoantipyrine, as it has been shown to be rapidly diffusible and also soluble in equal amounts in various tissues (1). In addition, iodoantipyrine has been found to reach a stable level in the brain of rats, within 6 sec after injection and to maintain this level for over a minute (13). It can therefore be used to estimate the relative distribution of cardiac output to paired cerebral hemispheres or optic lobes; 2-(^{14}C)-barbituric acid, which is also freely soluble in cerebral tissues, was used as an additional means of estimating blood flow.

Results

Preliminary experiments of regional cerebral blood flow in unoperated chicks, revealed that the circulatory system within the brain was essentially symmetrical, paired cerebral regions receiving the same amount of blood-borne isotope (Table 1). The specific activities of optic lobes and cerebral hemispheres from the same bird, were similar indicating that the vascular supply to these regions was equivalent.

At various times after enucleation, both cerebral hemispheres and optic lobes contralateral to the removed eye exhibited a reduced velocity of blood flow relative to the ipsilateral lateral regions (Table 2). Regions contralateral to the treated eye are referred to as deprived (D) while regions

TABLE 1

SYMMETRY OF VASCULAR SUPPLY TO PAIRED REGIONS OF CHICK BRAIN [a]

Region	L/R
Cerebral Hemispheres	1.02 ± 0.05
Optic Lobes	1.02 ± 0.03

[a] Ratio of counts/min/mg tissue for left (L) and right (R) brain regions, 10 sec after intracardiac injection of ^{125}I-iodoantipyrine. Eight chicks were used.

contralateral to the unimpaired eye are called visual (V). This asymmetrical vascular distribution appeared within an hour of eye removal and persisted for at least 6 days—the longest period examined. The effect was thus not confined to regions that are directly innervated by the optic tract, such as the optic tectum, but was apparent in cerebral cortical regions which receive very few direct fibers from the optic nerve (4).

Parallel studies on blood flow after monocular suture also revealed a rapidly developing differential between visual and deprived cerebral regions (Table 3). The asymmetry observed after monocular eyelid suture was less marked than that consequent to enucleation but was highly significant within an hour of suture.

Similar studies using ^{14}C-barbituric acid confirmed the data obtained with ^{125}I-iodoantipyrine, but had a somewhat greater variance. This was attributed to the assay of ^{14}C being more susceptible to quenching than ^{125}I.

Regional Plasma Volume. Determinations of regional plasma volume were made 1 hr after unilateral eye removal or eyelid suture. After either of these procedures, a significant differential was observed between paired chick optic lobes (Table 4). However in no case was a significant asymmetry observed between cerebral hemispheres. Any differentials between

TABLE 2

BLOOD FLOW IN CHICK BRAIN REGIONS AT VARIOUS TIMES AFTER UNILATERAL ENUCLEATION [a]

Time after enucleation	No.	Cerebral hemis.		Optic lobes	
		V/D	p	V/D	p
1 hr	8	1.15 ± 0.04	0.025	1.35 ± 0.07	0.005
1 day	8	1.12 ± 0.04	0.05	1.16 ± 0.07	0.05
6 day	7	1.15 ± 0.07	0.05	1.09 ± 0.04	0.05

[a] Ratio of counts/min/mg tissue for visual (V) and deprived (D) chick brain regions, 10 sec after intracardiac injection of ^{125}I-iodoantipyrine into chicks that had been previously unilaterally enucleated. p = probability of ratio being significantly greater than unity. No. = number of chicks for each experimental point.

TABLE 3

BLOOD FLOW IN CHICK BRAIN REGIONS AT VARIOUS TIMES AFTER MONOCULAR EYELID SUTURE [a]

Time after suture	No.	Cerebral hemis.		Optic lobes	
		V/D	p	V/D	p
1 hr	8	1.16 ± 0.04	0.01	1.13 ± .06	0.05
1 day	7	1.03 ± 0.01	0.01	1.05 ± .02	0.05
6 day	8	1.08 ± 0.04	0.05	1.07 ± 0.03	0.05

[a] Ratio of counts/min/mg tissue for visual (V) and deprived (D) chick brain regions, 10 sec after intracardiac injection of ^{125}I-iodoantipyrine into chicks that had been previously monocularly sutured. p and No. = as for Table 2.

visual and deprived regions could not be related to capillary proliferation in this time interval (1 hr), but must rather have been a reflection of changes in vascular bed volume due to differences in capillary usage.

The observed decrease in regional blood flow following visual deprivation may at least in part be attributed to a reduction of total plasma space within specific cerebral regions. The number of actively functioning cerebral capillaries may vary with physiological state (11).

Discussion

The brain has a continuously high degree of oxidative metabolism and thus a great dependence on an uninterrupted and sufficient supply of oxygen and exogenous nutrients. This makes the adequacy of cerebral blood supply of initial importance. In man, cerebral ischemia very rapidly results in neurological malfunction.

The extent of brain development and the level of functioning ultimately attained may be related to the adequacy of cerebral blood supply. The data

TABLE 4

PLASMA VOLUME OF CHICK BRAIN REGIONS ONE HOUR AFTER UNILATERAL EYE REMOVAL OR EYELID SUTURE [a]

Treatment	No.	Cerebral hemis.		Optic lobes	
		V/D	p	V/D	p
Enucleated	18	1.10 ± 0.07	NS	1.29 ± 0.11	0.025
Sutured	28	0.97 ± 0.03	NS	1.09 ± 0.03	0.05

[a] Ratio of counts/min/mg tissue for visual (V) to deprived (D) chick brain regions, 90 sec after intracardiac injection of ^{125}I-albumin into chicks that had been monocularly treated 1 hr previously. p and No. = as for Table 2.

presented here show that this supply may vary in response to alteration of nervous afferentation. Interference with visual neuronal input can rapidly cause vascular deficits in the brain. These deficits are not confined to areas directly innervated by the eye, but appear to be widespread throughout secondarily innervated areas within the cerebral hemispheres.

Unilateral eye removal results in the total cessation of visual input to a single optic tectum. This has been shown to cause major developmental failure of the tectum and lesser metabolic effects in the corresponding cerebral hemispheres (2, 3). It is difficult to determine the relation between such impaired metabolism and reduced vascular supply, as both effects appear very rapidly after eye removal. Whether local cerebral circulation is largely governed by vascular innervation or by neurohumoral diffusion is also not known. Both phenomena may occur in the normal brain (6).

As the eyelid of the chick is very thin, it's suture does not prevent the passage of light to the retina of the eye. However, the light is nonpatterned and thus sensory input to the corresponding optic lobe is poor in information. It appears, then, that the removal of complex visual afferent information causes a decreased flow of blood within the primary visual area (optic lobe) and the secondarily innervated, associative area (cerebral cortex). This effect is restricted to one-half of the brain in monocularly treated birds and thus cannot be due to systemic variations of blood pressure.

Roy and Sherrington (12) were the first to introduce the concept of an intrinsic control of cerebral circulation and suggest that this was focally adapted to the regions metabolic and functional needs. A clear illustration of this is the increased local blood flow through the vascular bed of the cat visual cortex following retinal illumination that has been described (14). Capillary permeability may also vary with the physiological state of the brain (9). An increase of cortical blood flow in human beings during mental effort has been found (5). However, as the authors point out, this increase may in part be due to the peripheral and autonomic effects of tension and arousal which could increase afferent cerebral flow.

A deficit of patterned information-rich sensory input may restrict cerebral circulation. If the deprivation is transient, this effect may be reversible. However, previous work suggests that a continuing absence of meaningful sensory afferentation during a period of growth may permanently restrict optimal brain development (2).

Some or all of this retardation may be attributed to a prolonged cerebral vascular insufficiency. Investigation of the reversibility of phenomena described here, is presently in progress.

This report indicates a mechanism whereby a complex sensory environment may be essential for the development and maintenance of optimal cerebral associative and integrative capacity.

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