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# The Nutrient Supply of the Chick Visual System: Responses to Form and Light Deprivation

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Blood flow and uptake of two nutrient analogues have been studied in the neural retina, pigment epithelium and optic lobes of 5–7-day-old chicks after systemic administration of isotopically labeled compounds.

The pigment epithelium accumulated the inert amino acid, α-aminoisobutyric acid, 6 times as effectively on a wet weight basis than did the neural retina or optic lobes. However, the neural retina was over 3 times as active in transport of deoxyglucose as was either the pigment epithelium or optic lobe. These differences could not be attributed to variations in blood flow since the penetrance of the freely diffusible antipyrine was low in the neural retina and roughly equal in the vascularized epithelium and optic lobes. Two days of unilateral eyelid suture significantly depressed blood flow and deoxyglucose uptake in the optic lobe innervated by the occluded eye. The neural retina of the eye receiving reduced light input also had a reduced level of deoxyglucose accumulation but the capacity to concentrate α-aminoisobutyrate was increased. The corresponding dark-adapted pigment epithelium had a major (48%) increase in the uptake of this inert amino acid. The data show that regional glucose utilization may not be directly proportional to blood flow or glucose diffusion rates. The non-vascularized neural retina possesses an unusually powerful glucose transport mechanism while the pigment epithelium has a high capacity for amino acid accumulation.

## INTRODUCTION

Visual deprivation, either by monocular occlusion or dark rearing, is known to alter the rates of blood flow and glucose consumption of brain regions, primarily or secondarily associated with the visual input of the optic nerve[7,14]. Less work has been reported on such parameters in the retina under various sensory conditions[1,20,28,30] although the effect of light intensity upon retinal morphology has been extensively studied[16,22].

In this work, we report the effect of monocular suture on the metabolic state of the neural retina and retinal pigment epithelium as judged by (1) rates of blood flow, (2) capacity to accumulate an inert amino acid, and (3) intensity of glucose utilization estimated by deoxyglucose transport.

Antipyrine has been frequently used to evaluate regional blood flow, in view of its rapid diffusibility through aqueous and lipid-rich media and its non-volatility[2,23,24]. Blood flow bears a relationship to metabolic and functional activity[26]. Deoxyglucose is taken up by the glucose transport system and phosphorylated within the cell. Deoxyglucose is very rapidly phosphorylated in the toad retina and is largely not metabolized beyond the initial phosphorylation[30]. In view of its ionic hydrophilic nature, it is relatively slowly transported out of the cell[27] and largely remains trapped at the site of transport.

α-Aminoisobutyrate (AIB) accumulation was used as a marker for the type A neutral amino acid transport system[10]. The rate of entry of amino acids into nerve tissue may be regulated by neuronal activity which may in turn serve to regulate protein synthesis[18]. While the blood–brain barrier initially retards the penetrance of α-aminoisobutyric acid (Schain and Watanabe, 1972) it is actively concentrated by brain tissue after several passages of the

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compound in the plasma[9]. This appears to be due to the combination of a low rate of transport across brain capillaries and a rapid concentrative uptake by brain cells[5].

The avian visual system was chosen for study because of several advantageous features: (1) large eye size relative to body weight allows a clear separation of the neural and pigment epithelial components of the retina; (2) the avian retina is thicker than the mammalian retina and is avascular so that capillary contamination of this dissected material is minimal[17]. Thus, all nutrients must cross the selective blood–retinal barrier through the pigment epithelium[13]; (3) the predominant sensory input of the bird is visual and the optic tecta constitute around 30% of brain weight. A relatively large proportion of total systemically administered isotope is thus present in these components. The total crossover of the optic chiasm causes each eye to solely innervate the contralateral optic lobe[11]; (4) use of internal controls reduces variance and makes small differences readily distinguishable.

## MATERIALS AND METHODS

### *Animals*

New hatched white Leghorn chickens were maintained in the usual cyclic light environment (12 h light/12 h dark) and housed together in heated brooders with free access to food and water. All experiments were conducted in ambient fluorescent lighting at least 3 h after the onset of the light cycle. At 3 days post-hatch eyes were sutured under light halothane anesthesia, after which chickens rapidly recovered (< 2 min). The physiological studies were carried out after 2 days of visual deprivation. At the conclusion of the experiment, chicks were decapitated, blood was collected and tissues were rapidly dissected out and weighed. Erythrocyte-free serum was obtained after the blood had coagulated.

### *Dissections*

Eyes were freed of adhering tissues, the eyeball was hemisected and the vitreous humor removed. A preweighed filter paper disc was then placed on the retina and the adhering neural retina could then be separated out effectively[20]. The pigment epithelium and choroidal tissue could then be lifted off. The performance of this procedure in the light results in a degree of cross-contamination due to interdigitation of the neural retina with the pigment epithelium. Portions of photoreceptor outer segments often remain with the apical microvilli of pigment epithelial cells[3,17].

### *Isotopically labeled compounds*

Blood flow was evaluted after intracardiac injection of 0.1 ml (10 $\mu$Ci) [N-methyl-$^{14}$C]antipyrine (53 mCi/mmol). Birds were decapitated 12 s after injection. Glucose utilization was estimated by intracardiac injection of 0.1 ml (10 $\mu$Ci) [1-$^{14}$C]2-deoxy-D-glucose (51.3 mCi/mmol) or [$^{3}$H(G)]2-deoxy-D-glucose (5 Ci/mmol). Animals were decapitated an hour later. The transport of $\alpha$-aminoisobutyric acid (AIB) was determined by decapitation of chicks 5 h after intraperitoneal injection of 0.1 ml (5 $\mu$Ci) of [2,3-$^{3}$H(N)]$\alpha$-aminoisobutyric acid (34.7 Ci/mmol). All isotopes were obtained from New England Nuclear Corp. (Boston, MA). After weighing, tissues were dissolved in 1 ml tissue solubilizer (NCS, Nuclear Chicago Co., Chicago, IL), at 60 °C after which 19 ml of standard POPOP + PPO scintillation fluid (Spectrofluor Nuclear Chicago, IL) were added. After 24 h, radioactivity was determined, results being corrected for quench by use of an external standard.

### *Data expression*

Results were calculated as cpm/g wet weight (or per ml volume in the case of serum). In the case of intracardiacly administered materials, abnormally low

TABLE I

*Tissue concentration of blood-borne chemicals, in retina and optic lobes of the chick*

All values were calculated as cpm/mg wet weight and are expressed relative to the corresponding value for serum. Each value represents the mean ± S.E. of the ratios derived from 7–12 birds. Further details are given in the Methods section.

| *Isotope* | *Neural retina* | *Pigment epithelium* | *Optic lobes* |
|---|---|---|---|
| Antipyrine | 0.34 ± 0.07* | 0.67 ± 0.12 | 0.80 ± 0.11 |
| Deoxyglucose | 4.59 ± 0.43* | 0.58 ± 0.11* | 1.54 ± 0.10* |
| α-Aminoisobutyrate | 0.59 ± 0.04 | 3.43 ± 0.37* | 0.60 ± 0.03 |

* Value differs significantly from that of any other region ($P < 0.05$; Analysis of Variance, Fisher's Least Significant Difference).

counts (< 400 counts/min/100 mg tissue) signified failure to inject the heart, and such data were rejected. Where data were expressed as E/C, being the counts found in the experimental (E) region relative to those in the control (C) region, the natural logarithm of this value was used in statistical computations in order to avoid skewing the data[6]. The accepted level of significance was taken as $P < 0.05$ using a two-tailed distribution with the Wilcoxon Signed Rank Test[29] for paired (E/C) data. When comparisons were made across different tissues, analysis of variance was performed followed by Fisher's Least Significance Test. Each data point presented was derived from 7–18 individual birds.

## RESULTS

### *Accumulation of blood-borne materials*

The rate of antipyrine penetrance into the neural retina was markedly lower than the values for pigment epithelium and optic lobe (Table I). This was consistent with the absence of capillaries in the neural retina of the bird. However the [$^3$H]deoxyglucose level of the neural retina, 1 h after intracardiac injection, was several-fold higher than values for other tissues or circulating plasma. In contrast, the largest capacity for AIB accumulation was found in the pigment epithelium, 5 h after intraperitoneal injection of this isotope (Table I). At this time, AIB was several-fold more concentrated in the pigment epithelium than in any other tissue examined.

### *Effects of eyelid suture*

After 48 h of unilateral visual occlusion, the contralateral optic lobe, receiving reduced sensory input, had a decreased rate of blood flow (Table II). This effect was detectable within 15 min of suture and, as previously reported[7], was rapidly reversible, showing an 'overshoot' phenomenon, 2 h after suture removal (Table II). The suture procedure had no discernable effect upon the penetration of antipyrine into the neural retina but blood flow in the pigment epithelial–choroidal layer was significantly increased (Table III). One hour after intracardiac injection of labeled deoxyglucose, the neural retina of the occluded eye had a reduced level of deoxyglucose accumulation relative to the corresponding untreated eye. This reduction paralleled that seen in the optic lobe innervated by the retina receiving only dim, unpatterned light (Table III).

TABLE II

*Effects of unlateral eyelid suture on blood in the optic tectum*

Animals were decapitated 10 s after intracardiac injection of antipyrine. Data are expressed as the ratio of cpm/mg tissue for each experimental region (E) relative to its contralateral paired control (C). Each point is derived from 6–10 individual birds.

| *Treatment* | *E/C* |
|---|---|
| 15-min suture | 0.88 ± 0.03* |
| 48-h suture | 0.87 ± 0.12* |
| 48-h suture, then unsutured for 2 more hours | 1.16 ± 0.09* |

* Experimental value differs significantly from control ($P < 0.05$, Wilcoxon Signed Rank Test).

TABLE III

*Effects of monocular occlusion upon accumulation of systemically administered compounds*

Data are expressed as the ratio of cpm/mg tissue for each experimental region relative to its paired contralateral control. Each point is derived from 7–18 individual birds. Details are given in the Methods section.

| *Isotope* | *Neural retina* | *Pigment epithelium* | *Optic lobe* |
|---|---|---|---|
| Antipyrine | 1.14 ± 0.16 | 1.33 ± 0.20* | 0.87 ± 0.03** |
| Deoxyglucose | 0.92 ± 0.04* | 1.13 ± 0.29 | 0.91 ± 0.02** |
| α-Aminoisobutyrate | 1.20 ± 0.07** | 1.48 ± 0.08** | 1.02 ± 0.02 |

* $P < 0.05$, **$P < 0.01$, experimental value differs significantly from control (Wilcoxon Signed Rank Test).

The most marked changes seen in the experimental retina were significant increases in the concentration of α-aminoisobutyric acid in both neural and pigment epithelial components, 5 h after intraperitoneal injection of this inert amino acid. This accumulation was most pronounced in the pigment epithelium, which also had a much higher basal level of AIB uptake than other tissues examined (Table I). The 5 h point did not allow full tissue equilibration of AIB as this may take at least 24 h[8].

## DISCUSSION

The high level of accumulation of deoxyglucose into the retina may reflect a major transcellular flux of glucose through the retinal pigment epithelium. This suggested the existence of a unidirectional carrier-mediated glucose transport mechanism across

the pigment epithelium. There is evidence for such transcellular transport[12,13,32]. It seems remarkable that an avascular region such as the neural retina has such a great capacity to accumulate deoxyglucose. This is in contrast to its AIB uptake capacity which does not differ from that of the optic lobe. This tissue contains few intercellular spaces but mostly zona occludens (tight junctions) between cells[13], so little intercellular movement is possible. However the retina can use glucose and amino acids derived from the vitreous humor[4]. This latter mechanism may be especially pronounced in birds which have an avascular retina and a highly vascular pecten protruding into the vitreous body[15]. Deoxyglucose uptake seems to reflect electrical activity within brain tissue[14] and such uptake in the retina is influenced by the membrane potential[1], being increased under depolarizing conditions.

The effect of light on retinal accumulation of deoxyglucose has been described as inhibitory, in contrast to our data. However, other reports have utilized in vitro systems[28] or total darkness[20,30]. Eyelid suture results in dim unpatterned light reaching the retina and this has markedly different effects on the visual system than does total darkness[21]. Studies involving total darkness can lead to alterations in levels of circulating hormones that can cause widespread metabolic changes. On the other hand, any differences observed between sutured and unsutured eyes from a single animal cannot be attributed to the endocrine system. Analysis at the cellular level has revealed that, in the dark-adapted retina, deoxyglucose uptake into photoreceptors is enhanced, while less isotope is taken up by bipolar and amacrine cells[1]. This may account for inconsistent data derived from studies involving tissues containing many cell species.

The pigment epithelium had a very high capacity to concentrate AIB (Table I) and this was further increased in the sutured eye (Table III). The pigment epithelium is most active in the phagocytosis and disposal of shed outer cone segments, during dark hours[31] and the increased AIB uptake in the pigment layer of the sutured eye may be related to this increased metabolic activity. The finding that the pigment epithelium concentrates AIB almost 6 times as much as the retina (Table I), suggests that the increase in neural retinal AIB within the sutured eye (Table III) could be an artifact due to minor contamination with pigment epithelium.

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