

VISUAL STIMULATION INCREASES REGIONAL CEREBRAL BLOOD FLOW AND METABOLISM IN THE GOLDFISH

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SUMMARY

Studies with radioactive antipyrine and deoxyglucose indicate that both blood flow and metabolism are enhanced in the goldfish optic tectum following a brief visual stimulation. It is concluded that the brain of cold-blooded animals demonstrates regionally regulated physiological alterations, previously reported only in warm-blooded species.

INTRODUCTION

The teleost has proved to be a useful source of neurobiological model systems. It manifests marked similarities and contrasts with the mammalian nervous system. For example, the finding in the goldfish¹ that inhibitors of protein synthesis have no effect on acquisition of new behavior but block formation of long-term memory had been amply confirmed in both avian⁵ and rodent models^{3,4}. On the other hand, while the adult goldfish and a number of other poikilotherms regenerate lesioned pathways within the central nervous system, this is not seen in birds or mammals⁶.

It is thus of considerable interest to note what other similarities and differences exist in brain physiology and biochemistry between cold-blooded and warm-blooded animals. A striking property of mammalian brain is the highly sensitive regional control of cerebral blood flow (CBF)^{7,17,26} and of energy metabolism²¹. The present study was performed to learn whether such regional changes in cerebral blood flow and energy metabolism could be demonstrated in teleosts. The goldfish visual system appeared suitable for such a study. Since the optic nerves are completely crossed^{10,18,19}, one might observe altered blood flow and metabolism in the contralateral tectum following unilateral stimulation, while the ipsilateral tectum could serve as a control tissue.

METHODS

Goldfish (*Carassius auratus*), 8–11 g, Ozark Fisheries, Stoutland, Mo., were maintained under constant illumination without feeding for several days in individual 0.5-gallon clear plastic tanks at 20 ± 1 °C. Tricaine methanesulfonate (MS-222, Finquel) was purchased from Ayerst Labs (New York, N.Y.), 2% lidocaine hydrochloride was obtained from McGaw Labs (Glendale, Calif.) and [1-¹⁴C]2-deoxy-D-glucose (DG), [G-³H]DG and [*N*-methyl-³H]antipyrene (AP) were obtained from New England Nuclear (Boston, Mass.)

Unilateral eye enucleation was performed under MS-222 anesthesia (50 mg/l of tank water) 24–48 h prior to light stimulation. The stimulus consisted of a white light source producing a Talbot luminescence of 1 ft L at 5 cm (10 msec duration, 5 Hz). Measurement of the light source was made with a SEI photometer. During the period of stimulation the fish were confined in a small compartment against the tank wall. Unless otherwise specified, 5 min following the onset of stimulation fish were injected with the isotopic tracer (DG, AP, or both). Stimulation was then continued for 5–90 additional min. At the end of the stimulation period, fish were quickly decapitated and both tecta were removed and stored at -70 °C. Each tectum was homogenized in 0.2 ml of H₂O and 20 μ l duplicate aliquots were taken for protein determinations¹¹. The remainder was applied to a filter paper disc, dried and combusted in a sample oxidizer (Packard 306) prior to determination of radioactivity by liquid scintillation spectrometry. Results are expressed as a ratio of specific activities (cpm/ μ g protein) of contralateral (stimulated) tectum to the deafferented, ipsilateral (unstimulated) tectum. Student's *t*-test for paired data was used to determine significance of the difference between samples.

For radioautography of the tectum with [¹⁴C]DG, the brain was frozen after the period of stimulation, and cryosections, 20 μ m thick, were applied to RP-X-Omat film (Kodak) according to the method of Sokoloff^{8,21,23}.

RESULTS

Effects of brief stimulation

The first experiment evaluated simultaneously effects on CBF and on glucose metabolism by means of a double-label approach, using [¹⁴C]DG to measure cerebral metabolism and [³H]AP to measure CBF. Because the time interval during which AP diffusion in the brain is rate-limited by CBF is brief¹⁴, the incorporation period was limited to 5 min. Following 5 min of light stimulation, unilaterally enucleated goldfish were injected intracardially with a mixture containing 5 μ Ci of [¹⁴C]DG (52.6 mCi/mmole) and 5 μ Ci of [³H]AP (157 mCi/mmole) in a total vol. of 20 μ l. The stimulus was continued for 5 additional min at which time the fish were decapitated and tecta removed and analyzed, as described in Methods. Significant increases in both [³H]AP and [¹⁴C]DG ratios (contralateral/ipsilateral) were observed as a function of stimulation (Table I). The apparent increase in CBF was 30%, while that in glucose utilization was 26%. In a similarly treated group of animals maintained in

TABLE I

Effects of visual stimulation on tectal CBF and glucose utilization

Previously unilaterally enucleated fish were injected with both [^{14}C]DG and [^3H]AP, as described in the text, and were killed 5 min later.

N	Condition	Contralateral (stimulated) / ipsilateral (unstimulated)			
		^{14}C ratio (\pm S.E.M.)	P	^3H ratio (\pm S.E.M.)	P
12	Light flash	1.26 \pm 0.04	0.01	1.30 \pm 0.03	< 0.005
5	Dark	1.10 \pm 0.12	n.s.	1.12 \pm 0.14	n.s.

the dark (except for a low-intensity red light to permit observation) the differences between the two tecta were not seen, suggesting that the observed increases were indeed a function of visual stimulation.

Effects of prolonged stimulation

Prior studies with DG and AP in mammals suggested that the differences in the DG distribution persist for long periods of time²³, while those in AP do not¹⁴. In rat brain, DG-6-P has a half-life of several hours²³, and incorporation periods of 30–60 min are commonly used. Regional AP distributions, however, are not seen following such long pulses. It was of interest therefore to learn whether the distinctly different time courses of labeled AP and DG distribution occurred in goldfish, since such a dissociation would confirm that the two tracers were reflecting altered CBF and glucose metabolism respectively. The results of such studies could bear on a number of alternative hypotheses, which might explain our initial findings.

The possibility that the present results were somehow a sequela of enucleation and consequent tectal deafferentation was investigated by means of a time course with AP. In view of our demonstration of differences of radioisotopic distribution in the two tecta indicated by the brief AP pulse, further demonstration of its disappearance with longer pulses would support the view that the phenomenon reflected CBF changes, and would argue against the hypothesis that a structural change in the AP space had occurred following enucleation. Unilaterally enucleated fish were treated as in the initial experiment, except that 5.5 μCi of [^3H]AP were injected at 5 min intramuscularly (i.m.) and stimulation was then continued for an additional 5, 15, 45 or 90 min. Significant differences between the two tecta were seen at 5 and 15 min, but were absent by 45 min (Table II). Although intracardiac injection used in the previous experiment is desirable for short pulses, the intramuscular route is generally more reliable, and is preferred for longer pulses.

The distribution of DG following a 45 min incorporation pulse was investigated. This longer pulse with DG was employed to ensure that the differences in metabolism noted with a 5 min DG-pulse represented actual metabolism (i.e. phosphorylation of DG to DG-6-P), and not simply free DG distribution in brain. In this experiment unilateral enucleation of the fish was not performed. Instead, fish were temporarily unilaterally blinded by intraocular injection of 5 μl of 2% lidocaine hydrochloride 10

TABLE II

Effect of duration of labeling pulse on AP distribution in optic tectum

Previously unilaterally enucleated fish were injected intramuscularly with [³H]AP and killed at various intervals as stated.

N	Duration (min)	Contralateral (stimulated) ipsilateral (unstimulated)	
		Ratio (\pm S.E.M.)	P
5	5	1.24 \pm 0.06	< 0.01
5	15	1.17 \pm 0.05	< 0.05
5	45	0.99 \pm 0.06	n.s.
5	90	1.01 \pm 0.06	n.s.

TABLE III

Effect of prolonged DG pulse and temporary xylocaine blindness on glucose utilization in tectum

Fish unilaterally blinded with 2% xylocaine were injected intramuscularly with [³H]DG, as described in text, and were killed 45 min later.

N	Condition	Contralateral (stimulated) ipsilateral (unstimulated)	
		Ratio (\pm S.E.M.)	P
10	Light flash	1.32 \pm 0.11	< 0.01
8	Dark	1.07 \pm 0.05	n.s.

min prior to visual stimulation²⁵. This local anesthetic was used to establish whether enucleation itself was responsible for the observed differences in DG-6-P distribution. Effectiveness of the injection was easily demonstrable by the appearance of postural asymmetry as a result of the dorsal light reflex¹². After 5 min of light stimulation, 7 μ Ci of [³H]DG (8.26 Ci/mmol) were injected bilaterally and the light stimulation continued for 45 additional min. Brains were then removed and processed as described in Methods. As in the 5 min pulse experiments, a significant increase in the DG ratio was seen in animals that had been stimulated, but not in control animals that had also been injected unilaterally with lidocaine and maintained in the dark during the 45 min incorporation pulse (Table III).

Radioautographic alterations in tectum following visual stimulation

Unilaterally enucleated goldfish were exposed to 5 min of light stimulus and then given 5 μ Ci of [¹⁴C]DG bilaterally. Stimulation was continued for 45 additional min in which the brain was removed, frozen and sectioned in a cryostat. Dried sections (20 μ m thick) were exposed to RP-X-Omat film for 6–10 days. The radioautographs (Fig. 1) show an increase in grain density over the stimulated (contralateral) tectum.

DISCUSSION

The use of antipyrine for the study of regional cerebral blood flow in the

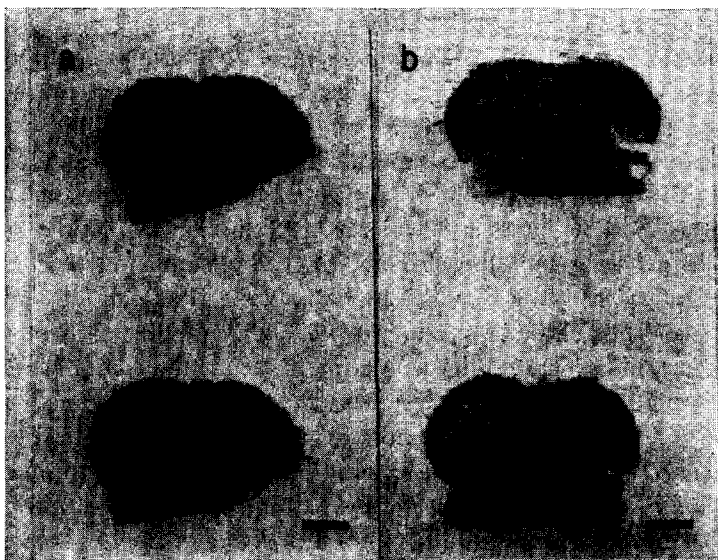


Fig. 1. [^{14}C]DG radioautograph of sequential $20\ \mu\text{m}$ sections through optic tecta of unilaterally enucleated goldfish (a) following 45 min of light stimulation and (b) control — no stimulation. Note increased density in the left optic tectum of the stimulated fish. Bars = 1 mm.

mammalian brain, particularly in connection with radioautography, has been known for some time. While AP is readily diffusible in brain, its distribution is limited by CBF for the first few minutes following its injection in a fashion analogous to that seen with inert gases¹⁴. While the period of time following injection during which AP can serve as an indicator of CBF may be as brief as 5 min in the cat, it appears that, following an i.m. injection in the fish, equilibration takes place between 15 and 45 min later. While it would require periodic blood sampling to quantify CBF by means of AP in the fish, the result does unequivocally demonstrate a significant increase in CBF in the visually stimulated tectum.

For the determination of regional energy metabolism in brain, the principle of a recently introduced radioautographic [^{14}C]DG technique was used^{8,23}. The method is based on the unique metabolic properties of DG, an analog of D-glucose, which is transported into brain cells and is phosphorylated by hexokinase, together with glucose. Unlike glucose-6-phosphate, however, DG-6-phosphate cannot be metabolized further, and is trapped intracellularly in amounts that are proportional to the local rates of glucose utilization during the uptake period. Using this marker for energy metabolism, a significant increase was noted in the visually stimulated tectum when compared to the unstimulated side. Since the goldfish is maintained in a 'normal' environment without anesthesia, prior to and during the experiment, the metabolic and blood flow changes observed are felt to represent a characteristic response to a physiologic stimulus of an intensity which the animal might encounter under natural living conditions.

While the small differences in the AP and DG ratios between the 'unstimulated' control and deafferented tectum in Table I do not reach a level of significance in the

present experiments, it is possible that they represent a small amount of visual stimulation in the darkened room. Initial concern in the 2-DG studies that the differences found upon visual stimulation were somehow more related to the enucleation than to the light stimulation was dispelled by the experiment in which we employed lidocaine anesthesia instead of eye removal. The 45 min pulse also assured that the differences in distribution of radioactivity noted at 5 min were indeed produced by metabolism, and not simply a reflection of DG distribution limited by blood flow. Further demonstration of the effect of light stimulation on glucose utilization in the brain is furnished by the radioautograph. While the use of ^{14}C -film radioautographs with the small fish brain results in considerably graininess, there is nevertheless a clear indication of increased incorporation in the tectum contralateral to the stimulated eye. The differences seen between the two sides are clearly a function of the light stimulation^{8,9} and not of deafferentation. Similarly, the time course with AP rules out the possibility that the brain AP space has been altered on the two sides as a result of the enucleation. The presence of a transient AP difference between the two tecta depends on light stimulation, and the result argues strongly that there has been a regional change in CBF as a result of the visual stimulation^{20,24}.

We thus conclude that visual stimulation in the goldfish leads to both increased CBF and glucose metabolism. Regional changes in CBF and in glucose metabolism may in fact be tightly coupled, as proposed in mammalian models^{2,13,15,16}, since increased glucose utilization may result in increased local pCO_2 , which can in turn lead to increased local CBF. Conversely, it can be reasoned that increased CBF brings increased glucose to cells, leading to increased utilization. While the present study does not address itself to a possible causal relationship between regional CBF and glucose metabolism, neither of these regional correlates of physiological function have previously been demonstrated in cold-blooded animals. In light of the present results, the techniques employed in this study may hold wide significance for studies in cold-blooded species in which the metabolic and circulatory correlates of functional activity are being investigated.

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