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Full Length Research Paper

Histochemical evaluation of the activities of glucose-6-phosphate dehydrogenase (G-6-PDH) and lactate dehydrogenase (LDH) in the visual relay centers of rat (*Rattus norvegicus*), bat (*Eidolon helvum*) and pangolin (*Manis tricuspis*)

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Activities of glucose-6-phosphate dehydrogenase (G-6-PDH) for the pentose phosphate pathway and lactate dehydrogenase (LDH) for the glycolytic pathway were compared in the rat (*Rattus norvegicus*), bat (*Eidolon helvum*) and pangolin (*Manis tricuspis*) visual relay centers to observe possible modifications that enable these mammals to cope with their habitation. Ten species each of both sexes were used for this study. After being sacrificed by cervical dislocation, their skulls were opened using bone forceps to expose the brains. The superior colliculi (SC) and lateral geniculate bodies (LGB) were excised from each brain, and were homogenized using Polter-Elvehjem homogenizer and assayed spectrophotometrically for the activities of G-6-PDH and LDH. It was observed that the histochemical activities of the enzymes showed significant differences ($p < 0.05$) of both SC and LGB of the three mammals as revealed in the quantitative histochemical localization of these two enzymes of carbohydrate metabolism. The result indicated that enzymes of carbohydrate metabolism play a vital role in the visual functions of these mammals.

Key words: Superior colliculus, lateral geniculate body, glucose-6-phosphate dehydrogenase, lactate dehydrogenase.

INTRODUCTION

The survival and reproductive capacity of any animal depends on its ability to allocate sufficient time and energy to a range of competing demands, such as foraging, maintenance, and reproduction (Schultz et al., 1999). Visual perception plays a role in the animal kingdom, most notably for the identification of food sources, suitable habitats, predators, and mate recognition in mating processes, as well as visual functioning to orient animals in their overall ecological surroundings (Cook, 1998).

In mammals, the visual pathways linking the eyes to the brain are those projecting to the lateral geniculate nucleus (LGB) and superior colliculus (SC) (Goodale and Milner, 2004; Kenneth, 2005). The lateral geniculate nucleus and the SC constitute the intracranial visual relay centres. While cells of the SC are involve in visual localization, movement and orientation of the eyes, accommodation and pupillary reflex, the LGB is the main thalamic centre for processing visual information by mediating vision and visual perception (Waleszczyk et al., 2007; Dale et al., 2007).

The rat, bat and pangolin are nocturnal mammals (Hildebrand and Goslow, 2001). The rat is omnivorous;

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bat is frugivorous, while pangolin is insectivorous. Rats are mammals with strong nocturnal ability. In rats, the lateral geniculate leaflet, participate in the regulation of circadian function through its projection to the circadian pacemaker of the hypothalamus. Bats are nocturnal and active at twilight (Fenton, 2001). Megabats have a well-developed visual cortex and show good visual acuity, while microbats rely on echolocation for navigation and food finding (Nancy et al., 2008). Pangolins are nocturnal, both ant- and termite-eating mammals and are active at night. They have limited vocal, visual and auditory acuity. Olfactory communication plays a significant role in their behavior (Feldhamer et al., 2007).

Glucose-6-phosphate dehydrogenase (G-6-PDH) is the first and rate limiting enzyme in the pentose phosphate pathway. G-6-PDH converts nicotinamide adenine dinucleotide phosphate (NADP+) into its reduced form, NADPH, and glucose-6-phosphate is then converted into a pentose sugar (ribulose-5-phosphate). The 5-carbon sugar is a precursor of DNA, RNA, and ATP (Peters and Van Noordan, 2009). Lactate dehydrogenase (LDH) catalyzes the conversion of pyruvate to lactate with concomitant oxidation of NADH during the last step in anaerobic glycolysis (Coquelle et al., 2007). It converts pyruvate, the final product of glycolysis to lactate when oxygen is absent or in short supply. G-6-PDH drives oxidative metabolism in the pentose phosphate pathway, while LDH drives anaerobic metabolism in the glycolytic pathway for ATP production.

This study was designed to comparatively evaluate the activities of G-6-PDH and LDH in the visual relay centers of rat, bat and pangolin.

MATERIALS AND METHODS

Animal care and experimental protocols

All experimental procedures followed the recommendations provided in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and Published by the National Institute of Health (NIH, 1985). Ten adult Wistar rats (*Rattus norvegicus*), ten fruit bats (*Eidolon helvum*), and ten pangolins (*Manis tricuspis*) of both sexes were used for this comparative study. The adult Wistar rats were obtained from the animal holdings of the Department of Anatomy of the University of Ilorin and were sacrificed shortly after purchase. The bats were curled down from their roosting colony at the flower garden area of Government Reserve Area (GRA), Ilorin; they were treated with tetracycline as prophylaxis against bacterial infection and sacrificed about 5 h later. The pangolins were procured from Asejire, a village in the North West Area of Osun State, Nigeria and were sacrificed before it was dark.

The animals were sacrificed by cervical dislocation. The skulls of the sacrificed animals were opened using bone forceps to expose the brain. The superior colliculi and lateral geniculate bodies were located via tracing of the optic tract to the optic chiasma and were excised with the aid of the atlas of the rat brain as described by Paxinos and Watson (2004). They were immediately transferred into separate cold 0.25 M sucrose solution and were homogenized with Polter-Elvhjem homogenizer as cited by Adekomi et al. (2011). The homogenate were centrifuged at 5000 rpm for 10 min as described in the study of David et al. (2009). The supernatants were immediately stored in the freezer (-20℃) and were assayed for the activities of G-6-PDH and LDH within 48 h by the methods of Lohr and Waller (1974) and Wei Bhaar (1975), respectively. The enzymes activities were read spectrophotometrically.

Statistical analysis

Data were reported as mean ± standard error of the mean (SEM). Significance was determined statistically by application of one-way analysis of variance (ANOVA) using statistical software (SPSS) version 17 at 95% confidence interval. Differences between means were considered statistically significant at $p < 0.05$.

RESULTS

Result obtained from the present study showed significant difference in the activities of the marker enzymes of the carbohydrate metabolic pathways (Tables 1 and 2).

DISCUSSION

Cortical structures such as the lateral geniculate nuclei and the superior colliculi have higher glucose utilization than other anatomical structures (Siesjo, 1978). Glucose utilization of these structures is achieved through carbohydrate metabolism. The difference between the metabolic potentials of the cells in different mammals adapting different environment cannot be over emphasized, with the contributions of the neurons and their supporting glial cells in these conditions. G-6-PDH and LDH are enzymes of carbohydrate metabolism that are involved in aerobic and anaerobic pathway, respectively for ATP production (Sodeinde, 1992). G-6-PDH and LDH therefore, are measure of ATP required for metabolism in the visual relay centres. However, the measure of G-6-PDH against LDH is of metabolic significance.

The cells used glycolysis as the primary source of energy. Increase in the activity of G-6-PDH indicates increased in carbohydrate metabolism for energy and ribose production via pentose phosphate pathway. From the result obtained, the rat utilizes more energy for visual movement and orientation. This is followed by pangolin and bat. The LGB of rat employs the largest amount of G-6-PDH activity; hence, is the most likely used in the largest quantity of energy to mediate vision and visual perception. This is followed by pangolin and lastly bat.

In over-exertion conditions when oxygen is absent or in short supply to cope with energy demands of the SC and LGB, LDH come into play in energy production. Increase in the activity of LDH indicates an increase in carbohydrate metabolism for energy production via glycolytic pathway. The measure of LDH in rat SC and LGB had the highest activity, followed by the bat and the least was observed in the pangolin.

Table 1. Activities of G-6-PDH in the visual relay centers of the three mammals (IU/L).

Animal	Superior colliculus	Lateral geniculate body
Rat	1191.66 ± 4.40*	1271.33 ± 21.21*
Bat	550.00 ± 9.07*	681.33 ± 21.17*
Pangolin	749.66 ± 23.96*	842.00 ± 14.01*

*Level of significance; SC= (G-6-PDH p = 0.000); LGB= (G-6-PDH, p = 0.000), (n=10).

Table 2. Activities of LDH in the visual relay centers of the three mammals (IU/L).

Animal	Superior colliculus	Lateral geniculate body
Rat	1420.00 ± 2.88*	1399.66 ± 4.91*
Bat	732.00 ± 20.22*	819.00 ± 4.72*
Pangolin	477.00 ± 28.98*	479.66 ± 17.57*

*Level of significance; SC= (LDH, p = 0.000); LGB= (LDH, p = 0.002), (n=10).

The feeding habits, lifestyle as well as the nature of their habitat would account for this. While rats are active during the day and night with higher mobility, they utilize their visual relay centers more than that of the bat and pangolin. The pangolins are only active at night. However, being an insectivore, they require highly effective vision to identify and contrast food which could be quite small, although, olfactory communication also plays a significant role (Feldhamer et al., 2007). The fruit bats on the other hand require less rapid movement of their eyes in search for food. They feed on fruits and depend on the combined use of echolocation and olfactory clues to find ripe fruits hidden and nestled among leaves (Korine et al., 2000; Mikich et al., 2003). Bat engages in true flight (Vaughan et al., 2000) and employs an alternative pathway for metabolism. This suggests why bat have a higher LDH activity level as compared to the pangolin. A closer look at the G-6-PDH:LDH in examining carbohydrate metabolism as a balance between aerobic and anaerobic metabolism in the animals studied shows that, in the SC, the rat's G-6-PDH:LDH ratio reads 1191.66 ± 4.40:1420.00 ± 2.88, indicating the anaerobic system is more active than the aerobic system, while the same was observed in the bats (550.00 ± 9.07:736.00 ± 20.22) with the levels of LDH being higher than those of G-6-PDH, indicating a negative shift in oxygen usage for metabolism, while a positive shift was seen in the pangolin with the ratio reading positive 749.66 ± 23.96:477.00 ± 28.98.

In the LGB, the rat's G-6-PDH:LDH ratio reads 1271.33 ± 21.21:1399.66 ± 4.91, indicating the anaerobic system is also more active than the aerobic system, while the same was observed in the bats with (681.33 ± 21.17: 819.00 ± 4.72) indicating a negative shift in oxygen usage for metabolism, while a positive shift was seen in the pangolin with the ratio reading positive 842.00 ± 14.01:479.66 ± 17.57, followed the same trend as in SC. The outcome of this study is in line with the observations of Adeniyi et al. (2012).

Conclusion

Data acquired from this study showed that the activities of the studied enzyme of carbohydrate metabolism (G6PDH and LDH) in the visual relay centres differ in the studied species of mammals. It was observed in rat and bat that the visual relay centres in these mammalian species utilizes more of the Embden-Meyerhof's pathway, while in pangolin the visual relay centres utilizes hexose monophosphate shunt. This suggests that the visual relay centres in the three mammalian species utilizes energy via different mechanisms for visual perception. This could be as a result of their individual peculiarities.

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REFERENCES

Adekomi DA, Ibiyeye RY, Popoola AN, Oyesomi OT (2011). Activities of G-6-PDH and LDH in the Kidney, Liver and Pancreas of Adult Wistar Rats following the Administration of Aqueous Leaf Extracts of Madagascar Periwinkle (*Catharanthus roseus*). Asian J. Cell Bio. doi: 10.3923/ajcb.2011. ISSN 1814-0068, pp. 1-8.

Adeniyi TD, Enaibe BU, Adekomi DA, Mohammed OA (2012). A comparative assessment of superior colliculus of Rat (*Rattus norvegicus*), Bat (*Eidolon helvum*) and Pangolin (*Manis tricuspis*): A Histochemical Study. Eur. J. Exp. Biol. 2(2):315-320.

Cook RG (1998). Visual perception. In G. Greenberg and M. Haraway (Eds). Encyclopedia of comparative psychology. New York: Garland publishing.

Coquelle N, Fioravanti E, Weik M, Vellieux F, Madern D (2007). Activity, Stability and Structural Studies of Lactate Dehydrogenases Adapted to Extreme Thermal Environments. J. Mol. Biol. 374:547-562.

Dale P, George JA, David F, William CH, Anthony-Samuel L, James OM (2007). Neuroscience, Fourth edition published by Sinauer Associates and Sumanas Inc.

David AJ, Adefolaju AG, Enaibe BU, Ajao MS, Caxton-Martins EA (2009). LDH and G6PDH Acivities in the ovaries of adult female Wistar rats following the administration of aqeoeus extracts of neem (Azadirachta indica) leaves. Afr. J. Biotechnol. 8(7):1310-1313.

Feldhamer GA, Lee CD, Stephen HV, Joseph FM (2007). Mammalogy, Adaptation, Diversity and Ecology Boston: WCB McGraw-Hill.

Fenton MB (2001). Bats. Checkmark Books, New York.

Goodale M, Milner D (2004). Sight unseen. Oxford University press, Inc: New York.

Hildebrand M, Goslow GE (2001). Analysis of vertebrate structure. 5th ed. New York, John Wiley and Sons Inc.

Kenneth LG (2005). Binocular visual responses in cells of the rat LGN.

J. Physiol. 566:119-124.
Korine C, Kalko EO, Herrel EA (2000). Factors affecting fruit removal and fruit characteristic in a community of strangler figs in panama. Oecologia 123:560-568.
Lohr GW, Waller HD (1974). Glucose-6-Phosphate Dehydrogenase. Methods of Enzymatic Analysis, 3rd Edition.
Mikich SB, Bianconi GV, Maia B, Teixera SD (2003). Attraction of the fruit-eating bat *Carollia perspicillata* to *Piper qaudichaudanium* essiential oil. J. Chem. Ecol. 29:2379-2383.
Nancy BS, Kevin LS, Jorg H, Gregg FG (2008). Primitive early Eocene bat from Wyoming and the evolution of flight and echolocation. Nature 451(7180): 818.
National Institutes of Health, NIH (1985). Guide for the Care and Use of Laboratory Animals: DHEW Publication (NIH), revised. Office of Science and Health Reports, DRR/NIH, Bethesda, USA.
Paxinos G, Watson C (2004). The rat brain in sterotaxic co-ordinates, 5th edition. Elsevier Academic Press.
Peters AL, Van Noorden CJF (2009). Glucose-6-phosphate Dehydrogenase Deficiency and Malaria: Cytochemical Detection of
Heterozygous G6PD Deficiency in Women. J. Histochem. Cytochem, 57(11):1003-1011.
Schultz LA, Collier G, Johnson DF (1999). Behavioural strategies in the cold: effects of feeding and nesting costs. Physiol. Behav. 67:107-115.
Siesjo BK (1978). Utilization of Substrates by brain tissues. Brain energy metabolism. John Wiley and Sons, USA.
Sodeinde O (1992). Glucose-6-phosphate dehydrogenase deficiency. Brailleire's Clin. Haematol. 5(2):367-382.
Vaughan T, Ryan J, Czaplewski N (2000). Mammalogy, 4th Edition. Toronto: Brook Cole.
Waleszczyk WJ, Nagy A, Wypych M, Berenyi A, Paroczy Z, Eordegh G, Ghazaryan, A, Benedek G (2007). Spectral receptive field properties of neurons in the feline superior colliculus. Exp. Brain Res. 181(1):87-98.
Wei Bhaar D, Grossau E, Faderal B (1975). Normal ranges of alpha-HBDH, LDH, AP and LAP as measured with substrate-optimated test charges. Med. Welt. 26:387-392.