

A BIPHASIC COMPARTMENTATION OF NEURO-TRANSMISSION ASSOCIATED METABOLIC PATTERNS DURING ELECTRO-INDUCED AXOPLASMIC TRANSPORT IN SHEEP MEDULLA OBLONGATA

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Summary : In the sheep medulla oblongata, on the induction of polarity by the applied voltage gradient of direct current along the length, the enzymes such as acetylcholinesterase and glutamate dehydrogenase showed anodal transport while the enzyme arginase showed cathodal transport indicating the possession of negative and positive charge densities on the enzymes. These studies indicated that the glutamate bound metabolism, one towards ammonia formation and the other towards the energy production and neural transmission, have opposed electro-characteristics. The acetylcholinesterase system had anodal characteristics coupled to the glutamate dehydrogenase patterns. The existence of two charge based compartmentation is envisaged in the neural tissue.

Key words : length wise polarization
cathodal and anodal transport

anodal and cathodal characteristics
charge based compartmentation

INTRODUCTION

The metabolism of glutamate in the brain was indicated to exist in different compartments where the oxidation or conversion of glutamate into γ -amino butyric acid (GABA) or ketoacids is shown to exist in different locations of the neural tissue (1, 23, 24). The investigations on the amphibian sarcoplasm (22) and the axoplasm of the medulla oblongata (19) have revealed the existence of dual metabolic system with emphasis on two types of charge characteristics. When the tissues are subjected to long axis voltage gradient, it was found that oxidoreductases system showed cathodal characteristics while proteolytic patterns appeared to exhibit anodal characteristics within the cytosol. Thus both the systems are found to co-exist within the cytosol of the tissues but differ in electro characteristics and respective metabolic activities, indicating the possibility of existence of two charge based compartments within the cytosol. This aspect had been considered and an attempt was made to investigate the possibility of the existence of compartmentation in the medulla oblongata, which is known to have continuous axonal tracts largely (4).

MATERIAL AND METHODS

Procurement and preparation of materials : Brains were obtained from medium sized healthy sheep after decapitation of animals, which were starved for 12 hrs and were separated by cutting anterolaterally from above the foramen magnum after removing the roof of the cranium. They were placed in a dry beaker kept in ice-cold thermosflask and washed repeatedly with Krebs–Hansleit ringer (11). The meningi were removed carefully, the medulla oblongata was isolated and sliced into two equal lateral halves. Out of them, one served as control while the other was subjected to voltage gradient of direct current, applied across two platinum electrodes of 0.2 mm diameter kept at 1 cm distance from each other on the tissue. The whole setup was placed in glass chamber, maintained at a temperature of 5°C to arrest the residual metabolism. The control tissue was not exposed to voltage gradient but was maintained in identical conditions.

Application of voltage gradient : The electrodes were connected to a regulated DC power supply unit and a voltage gradient of 5 volts DC/Cm was applied for a period of 10 min. This applied voltage gradient was found to be optimal in the earlier studies (18). Immediately after the exposure, the experimental as well as the control medulla oblongata were sliced transversely into six equal segments.

Cell free extracts : 10% (w/v) tissue homogenates were prepared in 0.25 M sucrose solution for the assay of glutamate dehydrogenase and in 0.1% cetyl trimethylammonium bromide for the assay of arginase respectively and centrifuged at 3000rpm for 15 min and the clear supernatant was used as enzyme source for the estimation of the activity levels. Separately, a 0.1% (w/v) homogenate of the tissue slices was prepared in sucrose and used without centrifugation for the assay of acetylcholinesterase.

Assay of enzymes : The assay of glutamate dehydrogenase (GDH L–glutamate: NAD oxidoreductase (deamination) EC 1.4.1.2) was done by the method of Lee and Lardy (12) using INT (2–p–iodophenyl–3–p–nitrophenyl–5–phenyl tetrazolium chloride) as proton acceptor. The assay of arginase (L–arginine amidino hydrolase EC 3.5.3.1) was done by the method of Beruter *et al.* (3) by determining the urea with diacetyl monoxime. The activity levels of acetylcholinesterase AChE, (Acetylcholine acetylhydrolase EC 3.1.17) (16) was estimated employing the method of Metcalf (16) and the protein content was estimated by the method of Lowry *et al.* (14) in the enzyme source.

RESULTS AND DISCUSSION

The values of the activity levels of the enzymes were plotted against the length of the long axis of medulla oblongata. The levels of the enzymes in the control slices

were found to be uniform along the length. Thus the activity levels in the control slices for GDH was $0.1322 \mu\text{mol}$ of formazan formed/ mg protein/ hr and for arginase it was $0.1057 \mu\text{mol}$ of urea formed/ mg protein/ hr , whereas for AChE it was $119.03 \mu\text{mol}$ of acetylcholine hydrolyzed/ mg protein/ hr (Fig. 1).

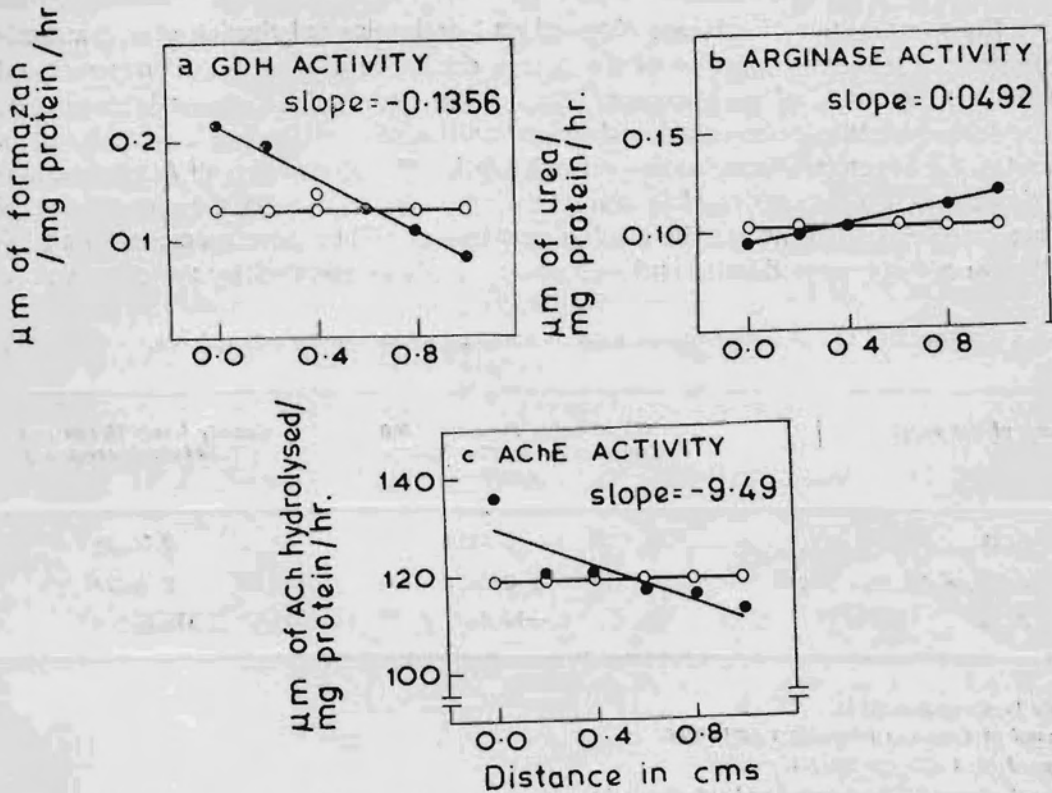


Fig. 1 : Activity levels of the enzymes, GDH, Arginase and AChE, represented along the length of the medulla oblongata polarized by long axis voltage gradient of 5 Volts DC/cm for 10 min.

When the tissues were subjected to induced electrogradient, the activities of the enzymes were found to vary in the cathodal and the anodal regions of the axoplasm. Thus GDH and AChE were found to show increased activity levels in the anodal axoplasm while arginase had shown increased activity levels in the cathodal segment of the axoplasm. The natural axoplasmic bidirectional transport of glutamate, cholinergic and GABA systems in medullary fibers and pons and their role in neuronal transmission in normal and pathological states have been reported in detail by earlier workers (8, 15, 21), suggesting the uniform prevalence of the associated metabolism. However in the present study there is selective electrotransport of AChE, GDH in the anodal and arginase in the cathodal direction suggesting the possibility of stepping up the specific metabolism

in the anode and cathode regions of the medulla oblongata. The increment in the cathodal axoplasm yields a positive slope for the curve, when computed, whereas an increase in the anodal axoplasm results in the negative slope as observed from the curves drawn in anode-cathodal axis (Fig. 1).

The slope values of enzymes obtained on length wise polarization of the medulla oblongata indicate the possession of the charge density in a comparative manner and also the activity levels of the enzymes. Greater the slope value, greater is the charge density (either positive or negative) and greater will be the activity levels of the enzyme. This relationship can be clearly envisaged in Table I. The slope value of AChE runs into two digits and the activity level is correspondingly higher. While the slope value of arginase is lowest and the activity level is also lowest. Thus there seems to be a relation between the charge density and the activity levels as revealed by the slope values.

TABLE I: The correspondance between the slope values and the activity levels.

<i>Name of the enzyme</i>	<i>Slope value (indicating the positive or negative charge densities)</i>	<i>Activity levels (Mean and \pmSD of six observations)</i>
GDH	— 0.1356	0.1322 ^a
Arginase	+ 0.0492	0.1057 ^b
AChE	—19.49	119.03 ^a

Activity levels expressed in

a - μ mol of formazan formed/mg protein/hr.

b - μ mol of urea/mg protein/hr.

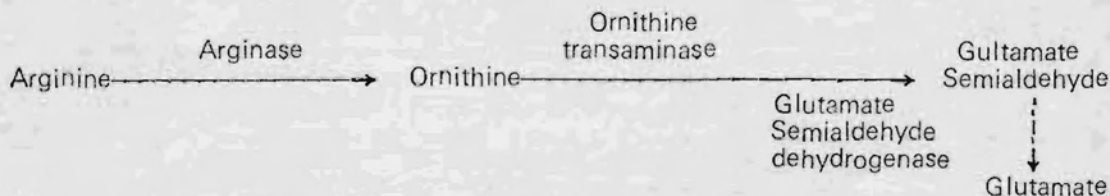
c - μ mol of acetylcholine hydrolyzed/mg protein/hr.

The negative slope value for GDH and positive slope value for arginase would reveal anodal and cathodal transports of the enzymes indicating opposed charge characteristics on them namely anodal and cathodal types. In the wake of these opposing characteristics, the implication of these two enzymes in glutamate metabolism is as follows.

The involvement of glutamate in the prevailing metabolism of axoplasm seem to be in two directions. One of the directions, evident in the anodal axoplasm, as seen in the high GDH activity, indicated the occurrence of oxidative deamination process leading to the formation of ammonia and 2-keto-glutarate. The free ammonia being toxic is converted into glutamine by the addition of ammonia unit indirectly into the gluta-

mate molecule. The increase in the glutamine content in anodal region has been reported by Mohanachari (17). Thus the anodal region is mostly involved in the metabolic processes associated with the ammonia production and the GDH possessing a net negative sign of charge. A second functional direction of glutamate is the promotion of activity in the neural tissues either by entering into the citric acid cycle oxidation processes thereby involving in the energy production or acting as neurotransmitter (6, 7, 10).

From the results, it is evident that the enzyme arginase undergoes cathodal transport in the polarized tissue indicating the possession of net positive sign of charge. This enzyme is known to be involved in ureogenesis from carbamyl phosphate units (9). But, since such full complement of urea cycle is reportedly lacking in neural tissue (2), the higher activity levels of this enzyme in the cathodal region may indicate the involvement of arginase in some other pathways. The formation of ornithine, expressed as a product of the enzyme activity, may indicate its further conversion to glutamic acid semi--aldehyde leading to the formation of glutamate (13).



Thus the cathodal characteristics of arginase catalysis indicated the possible synthesis of glutamate and its involvement in energy production and neural transmission. It is also known that the oxidoreductases of Krebs Cycle undergo cathodal transport indicating the possession of positive charge densities (20) and the involvement of glutamate in these oxidations is also known to be extensive by way of 2-ketoglutarate production. Thus the glutamate based energy production of transmission processes seem to possess cathodal characteristics. In contrast to this, AChE showed anodal mobility with anodal characteristics indicating the possession of negative charge density within the axoplasm.

Thus the cholinergic systems and ammonia metabolism in the axoplasm have anodal characteristics while the glutamate dependent transmitter activities and the energy production systems have cathodal characteristics. Likewise, within the axoplasmic framework, a type of metabolic compartmentation is envisaged, one compartment with anodal characteristics and the other with cathodal characteristics. It has to be seen whether the increase in any one of these characteristics artificially by imposing respective polarity can bring about a regulation in the activities of corresponding metabolic systems associated with the transmitter substances like glutamate and acetylcholine in the neural tissues.

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