Correlation of Sodium-Potassium-Activated Adenosine Triphosphatase with Acetyl Cholinesterase of the *Electrophorus* Electric Organ[1]

The electric organ of the electric eel (*Electrophorus electricus*) is a rich source of sodium-potassium-activated adenosine triphosphatase (Na^+-K^+-ATPase)[2–4], acetyl cholinesterase (AChE)[5] and choline acetylase (ChAc)[5]. Studies by NACHMANSOHN and his colleagues[6,7] have demonstrated that there is a gradient of AChE activity along the length of the electric organ, being highest rostrally and lowest caudally. This gradient of enzyme activity correlates with the density of electroplaques (cells) and the amplitude of the electric potentials[6,7].

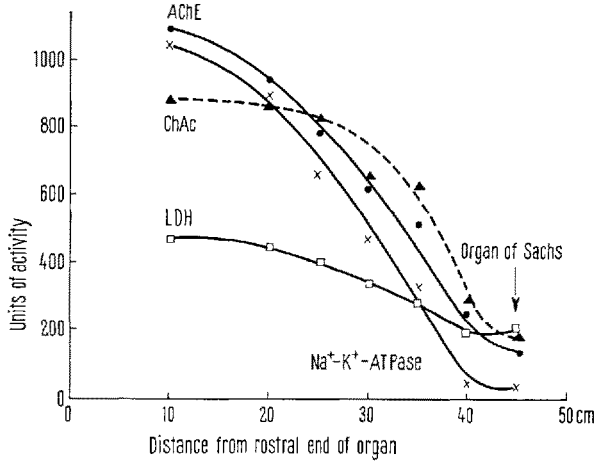
The caudal surface of each electroplaque is an innervated and conducting membrane. In contrast, the rostral membrane is not electrically excitable, has more invaginations and has a greater concentration of Na^+-K^+-ATPase activity[2], which has been implicated in the active transport of Na^+ and K^+ across cell membranes[8]. One test for the localization of this enzyme to surface membranes is to demonstrate that there is a gradient of Na^+-K^+-ATPase along the length of the electric organ similar to that found for AChE, which has been shown to be localized to electroplaque membranes[9]. As a control, a typical intracellular, soluble enzyme, such as lactic acid dehydrogenase (LDH), was also assayed.

Experimental. Tissue preparation. Electric eels were kindly provided by Dr. D. NACHMANSOHN. The fish were chilled in ice water and then sacrificed by decapitation. All further procedures, including centrifugations, were carried out at 4 °C. The electric organs, bilaterally, were exposed by dissection, and samples of tissue (0.8–4.0 g) were removed along the length of the main organ and the organ of Sachs. The samples were frozen immediately in a dry-ice freezer and stored up to 12 days at −70 °C. For the distribution studies the tissue samples were weighed and homogenized with 9 volumes of ice-cold water in a micro-blender (Eberbach Corp.) 5 times for 30 sec each time. For the subcellular fractionation studies the tissue was blended in 0.25 *M* sucrose instead of water.

Subcellular fractionation. After the homogenate was centrifuged at 1000 *g* for 10 min, the supernatant was decanted and the pellet washed with sucrose and recentrifuged. The pellet was resuspended in sucrose and labeled the nuclear (NUC) fraction. The pooled supernatants were centrifuged at 10,800 *g* for 10 min and the pellet was washed once and recentrifuged. This pellet was resuspended in sucrose and labeled the mitochondrial (MIT) fraction. The pooled supernatants were centrifuged at 190,000 *g* for 1 h. The pellet was resuspended in sucrose and labeled the microsomal (MIC) fraction. The remaining supernatant (SUP) fraction was also assayed.

Assay methods. For Na^+-K^+-ATPase determinations, 10 µl of each homogenate were diluted with 190 µl water and 5 µl 0.1 *M* EGTA[10] (neutralized to pH 7.0 with *Tris* base). Incubations were carried out at 26° for 30 min in a final volume of 25 µl. Each tube contained 5 µl of the diluted homogenate, 3 m*M* $MgCl_2$, 5 m*M* Na_4ATP, 60 m*M* NaCl, 24 m*M* KCl, and 51 m*M* *Tris*-HCl, pH 7.5. Half of the tubes also contained 10^{-3} *M* ouabain. The reaction was stopped with the addition of 200 µl of reagent, according to the method of LOWRY et al.[11], and the inorganic phosphate was measured spectrophotometrically. The Na^+-K^+-ATPase activity, which is the ouabain-sensitive ATPase, was calculated as the difference between the 2 sets of tubes, with and without ouabain. ChAc was assayed as described by MCCAMAN and HUNT[12] with slight modifications[13]. AChE was determined by the method of ELLMAN et al.[14]. The very high AChE activity in the electric organ required a 1000-fold dilution of each homogenate with 0.2% bovine serum albumin in 0.1 *M* PO_4 buffer, pH 8.0.. LDH was determined by the method of KORNBERG[15]. The protein content was measured by the method of LOWRY et al.[16].

Results and discussion. The average activities of Na^+-K^+-ATPase, ChAc, AChE, and LDH along the length of the electric organ bilaterally are shown in the Figure. The activities of Na^+-K^+-ATPase and AChE parallel each other very closely, both decreasing rapidly in a caudal direction. The activity of ChAc also is highest rostrally,



The correlation of enzyme activities along the length of the electric organ. The electric eel was 93 cm long and the rostral end of the electric organ began 20 cm behind the snout. The main organ was sampled from 10 to 40 cm from its rostral end, and the organ of Sachs, 45cm. Units of activity: ●——● AChE, µmoles acetylthiocholine (AcSCh) hydrolyzed min^{-1} g $tissue^{-1}$; ×——× Na^+-K^+-ATPase, µmoles Pi h^{-1} g $tissue^{-1}$; ▲——▲ ChAc, mµmoles ACh h^{-1} g $tissue^{-1}$; □——□ LDH, 10^{-7}moles NAD^+ min^{-1} g $tissue^{-1}$.

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but remains at a plateau until midway along the length of the organ, at which point the activity drops steadily. These 3 enzymes correlate best with the density of electroplaques and membranes, which have been shown to have similar gradients[6,7].

The activity of LDH tends to decrease along the length of the organ, but slopes more gradually than the other enzymes. Subcellular fractionation studies (Table) reveal that the latter enzyme is soluble and not membrane bound. Although the density of cells along the length of the organ decreases rapidly in a caudal direction, the cells become larger in size near the caudal end[7]. In fact, the cells are sufficiently large in the organ of Sachs to allow measurements on single cells[17,18]. Therefore, along the length of the electric organ, there is a smaller decrease in the intracellular content compared to the decrease in the cellular and membrane density. The distribution of LDH resembles that of succinic dehydrogenase[7] and both enzymes probably reflect intracellular content along the electric organ.

The organ of Sachs contains the lowest activities of all 4 enzymes studied. Calculation of the enzyme activities in terms of protein concentration yielded similar qualitative results. This study also demonstrates that the rostral part of the main organ is the preferable region in the preparation of Na^+-K^+-ATPase, AChE and ChAc from electric eels.

The specific activities of 3 enzymes from the subcellular fractionation of the main organ of *Electrophorus electricus*

Fraction	Na^+-K^+-ATPase μmoles Pi h-mg prot.	AChE μmoles AcSCh hydrolyzed min-mg prot.	LDH μmoles NAD^+ min-mg prot.
NUC	33.0	23.4	0.55
MIT	39.3	25.5	0.53
MIC	59.4	64.0	0.56
SUP	0.56	4.7	5.84

The assay methods were as described in the text with the exception that the various fractions were appropriately diluted to yield uniform results.

Résumé. La distribution des activités de la Na^+-K^+-ATPase, de l'acétylcholinestérase et de la cholineacétylase correspond à la densité des membranes le long de l'organe électrique. La plus haute concentration des activités enzymatiques ainsi que le plus grand nombre de membranes se trouvent dans la partie rostrale et tous 2 diminuent vers la partie caudale. La diminution de l'activité de la déshydrogénase lactique est plus graduelle dans le sens rostro-caudal et ceci correspond probablement au contenu intracellulaire le long de l'organe électrique. C'est dans l'organe de Sachs que l'activité des 4 enzymes etudiées est la plus basse.

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Stimulation und Depression der Phagozytoseaktivität des retikulo-endothelialen Systems nach Röntgenbestrahlung

Die Phagozytoseaktivität des retikulo-endothelialen Systems (RES) nach Ganzkörper-Röntgenbestrahlung (GKB) ist nicht geklärt. Mit verschiedenen Methoden wurden entweder keine oder nur geringe, insignifikante Veränderungen gefunden (Literatur bei[1]). Mit dem Kohle-Test (carbon clearance[2]) haben wir einen starken Phagozytose-Anstieg bei Mäusen festgestellt[3]. Zur gleichen Zeit berichteten Fred et al.[4] über eine Depression; sie haben ebenfalls Mäuse und den Kohle-Test benutzt, aber höhere Kohledosen angewandt. Es erschien deshalb notwendig, die Frage der Phagozytoseänderungen nach GKB nochmals eingehend zu prüfen.

Methodik. Weisse Mäuse, NMRI. 90 R/min; 200 kV, 15 mA. Bestimmung des Phagozytose-Index K und des korrigierten Phagozytose-Index α nach i.v. Injektion einer Kohlesuspension[2]. In einer ersten Versuchsreihe wurden nach Biozzi[2] 8 oder 16 mg Kohle/100 g Körpergewicht injiziert, in einer zweiten Versuchsreihe (Modifikation nach Fred[4]) 45 mg Kohle/100 g Körpergewicht.

Versuchsergebnisse. (I) Stimulation (8–16 mg Kohle pro 100 g): (1) Bei 30 Tage alten Mäusen stiegen die Werte für K und α am 4. Tag nach 750 R (DL 90/30) stark an und blieben bis zum 10. Tag erhöht (Tabelle). Das Maximum des Anstiegs lag zwischen dem 4. und 7. Tag. (2) Der Phagozytose-Anstieg war schon nach 250 R nachzuweisen. Er war zwischen 250 und 750 R dosisabhängig. 750 R und 1000 R zeigten keinen Wirkungsunterschied. (3) Die Strahlenwirkung war altersabhängig: der Anstieg trat bei 45 und 60 Tage alten Mäusen 2 bis 3 Tage später auf als bei 30 Tage alten Tieren. (4) Der Phagozytose-Anstieg schien um so grösser zu sein, je stärker die klinischen Symptome der Strahlenkrankheit ausgeprägt waren. (II) Depression (45 mg Kohle/100 g): Nach 750 R war die Phagocytose vom 1. bis 4. Tag herabgesetzt. Am 2. und 3. Tag war die Depression signifikant: unbestrahlte Kontrolltiere K = 0,0082, bestrahlte Tiere K = 0,0056 (je 10 Mäuse, Mittelwerte).

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