Purification, characterization and cellular localization of 5'-nucleotidase from *Torpedo* electric organ

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5'-Nucleotidase was isolated from the electric organ of the electric ray *Torpedo marmorata* after solubilization in Triton X-100 and deoxycholate by affinity chromatography on concanavalin A-Sepharose and AMP-Sepharose. The purified enzyme has a K_m for AMP of 38 μM , with a maximal velocity of 31 units/mg of protein. Of the purine and pyrimidine mononucleotides, AMP is hydrolysed most effectively. β -Glycerophosphate, phosphoenolpyruvate and *p*-nitrophenyl phosphate are not substrates for the enzyme. Adenosine 5'-[α,β -methylene]diphosphate, ADP and ATP are competitive inhibitors in this order of potency. Concanavalin A inhibits enzyme activity in a non-competitive manner. Whereas Mg^{2+} , Ca^{2+} and Sr^{2+} activate enzyme activity in the millimolar range, Hg^{2+} , and in particular Pb^{2+} and Zn^{2+} , inhibit enzyme activity. On SDS/polyacrylamide-gel electrophoresis the enzyme has an apparent M_r of 62000, whereas that of the native deoxycholate-enzyme complex is 131000. An antiserum raised against the native enzyme inhibits enzyme activity. Inhibition studies suggest the presence of tissue-specific variants of the enzyme. By immunohistochemical analysis the enzyme can be localized to the ramifications of nerve terminals in the electric organ.

INTRODUCTION

5'-Nucleotidase (EC 3.1.3.5), an intrinsic membrane glycoprotein of plasma membranes of a variety of cell types (see Karnovsky, 1986; Baron *et al.*, 1986), is contained in the electric organ of the electric ray *Torpedo marmorata*, a model system for the study of cholinergic synaptic transmission. On subcellular fractionation of the electric organ, 5'-nucleotidase becomes enriched in a fraction containing isolated nerve endings (synaptosomes). It behaves as an ectoenzyme, with its active site facing the extracellular medium (Grondal & Zimmermann, 1986). Similarly, ectoenzyme activities for the hydrolysis of ATP and ADP are associated with the synaptosomal fraction. This is of physiological significance, since the cholinergic nerve terminals store and release ATP together with the classical neurotransmitter substance acetylcholine, and the ectoenzymes are likely to be involved in the degradation of the released ATP to adenosine (Grondal & Zimmermann, 1986).

In order to describe the biochemical properties and the localization of the enzymes involved in the hydrolysis of extracellular nucleotides at the cholinergic synapse, they need to be isolated in pure form. We report here the purification and biochemical characterization of the 5'-nucleotidase from the electric organ of *Torpedo marmorata*. By use of a monospecific antiserum we localize the enzyme activity mainly to the nerve-terminal ramifications in the tissue.

EXPERIMENTAL

Materials

Media for chromatography (concanavalin A-Sepharose, AMP-Sepharose, Sephacryl 200) and the calibration kit for gel chromatography were obtained from Pharmacia. DEAE-Affi-gel was from Bio-Rad. Nucleo-

tides and phosphates (all sodium salt), lectins, α -methylmannopyranoside, deoxycholate, the calibration kit for SDS/polyacrylamide-gel electrophoresis and anti-rabbit IgG (FITC-conjugated) were purchased from Sigma. Triton X-100 was obtained from Serva, and [^3H]AMP and ^{125}I -labelled anti-rabbit IgG were from Amersham. All other reagents were of the highest grade available.

Enzyme purification

5'-Nucleotidase was purified from the electric organ of the electric ray *Torpedo marmorata* by a modification of the methods of Dornand *et al.* (1978) and Dieckhoff *et al.* (1985). All procedures were carried out at 4 °C unless otherwise stated.

Electric organ (100 g) was homogenized for 1 min in a Waring Blendor (high speed). To the homogenate 200 ml of 20 mM-Hepes/NaOH buffer, pH 7.4, was added, and the suspension was centrifuged at 3700 g (r_{av} 8.3 cm) for 10 min. The supernatant was re-centrifuged for 1 h at 35000 g (r_{av} 8.3 cm). The pellet fraction (P_1) obtained was resuspended in 50 ml of 1% (w/v) Triton X-100 in 20 mM-Hepes/NaOH, pH 7.4 and stirred for 10 min at room temperature. After centrifugation for 1 h at 35000 g (r_{av} 8.3 cm), the pellet (P_2) was resuspended in 50 ml of 5% Triton X-100 and 2% (w/v) deoxycholate in buffer A (0.4 M-NaCl, 20 mM-Hepes/NaOH, pH 7.4, 1 mM-MgCl₂, 1 mM-CaCl₂, 1 mM-MnCl₂). This suspension was again homogenized by two strokes in a Potter-Elvehjem homogenizer. The homogenate was incubated at room temperature for 1 h with continuous stirring. After a final centrifugation for 1 h at 35000 g (r_{av} 8.3 cm), the supernatant fraction (S_3) was stored overnight at -20 °C.

The frozen extract was thawed and loaded on a concanavalin A-Sepharose column (10 ml bed volume), equilibrated with 0.1% Triton X-100 in buffer A at room temperature (flow rate 30 ml/h). The column was washed

with 0.1% Triton X-100 in buffer A (50 ml), and the enzyme activity was eluted at a flow rate of 10 ml/h with 0.4 M- α -methyl mannoside/0.1% Triton X-100 in buffer A; 2 ml fractions were collected and stored overnight at -20°C . Fractions from concanavalin A-Sepharose chromatography containing 5'-nucleotidase activity were thawed and applied to an AMP-Sepharose column (2 ml bed volume) equilibrated with 0.1% Triton X-100 in buffer A at a flow rate of 10 ml/h (at 4°C). The column was washed with the same buffer (20 ml) and enzyme activity was eluted with 10 mM-AMP and 0.1% Triton X-100 in buffer A. The fractions containing 5'-nucleotidase activity were pooled and dialysed against 0.1% Triton X-100 in 20 mM-Hepes/NaOH buffer, pH 7.4, containing 10% (v/v) glycerol.

Isolation of synaptosomes

Synaptosomes were isolated from the electric organ as described by Grondal & Zimmermann (1986).

Analytical procedures

5'-Nucleotidase activity was routinely determined by analysis of P_i liberated after addition of AMP (0.5 mM), by the method of Lanzetta *et al.* (1979). The reaction was stopped by addition of trichloroacetic acid (final concn. 5%, w/v). Samples were then centrifuged for 10 min at 12000 g (r_{av} 6.0 cm) (4°C) and the supernatant was assayed for P_i . For analysis of kinetic properties of 5'-nucleotidase, a radiochemical assay was used as described by Gentry & Olson (1975).

Protein was determined as described by Peterson (1977), with bovine serum albumin as a standard.

Electrophoretic techniques

Polyacrylamide-gel electrophoresis in the presence of SDS was carried out as described by Laemmli (1970), on mini-gels (thickness 1.0 mm) with acrylamide concentrations of 5% (w/v) for the stacking gel and 10% (w/v) for the running gel. Silver staining was performed as described by Koepsell *et al.* (1984). Proteins were transferred from SDS gels to nitrocellulose as described by Towbin *et al.* (1979). The subsequent immunodetection is described in detail by Volknandt *et al.* (1987).

Gels were stained with peroxidase-labelled concanavalin A as described by Hawkes (1982).

Gel filtration on Sephacryl 200

For determination of the native M_r of 5'-nucleotidase, gel filtration on a column (1.4 cm $\times$ 50 cm) of Sephacryl 200 was performed in the presence of 0.6% deoxycholate. Aldolase, bovine serum albumin, ovalbumin and RNAase were used for column calibration.

Production of antisera

Antisera were raised in rabbits (New Zealand White) by injecting 100 μg of antigen, followed by three boost injections with 50 μg of antigen each. Native antigen was prepared by concentrating the dialysed enzyme by means of a Millipore immersible ultrafiltration unit (CX-30). For the preparation of denatured antigen, the purified enzyme was precipitated by the addition of 0.015% deoxycholate and 10% trichloroacetic acid (final concentrations) and resuspended in phosphate-buffered saline (150 mM-NaCl/2.5 mM-KCl/10 mM-sodium phosphate buffer).

All antisera were complement-inactivated and purified

by a 35% -satd.-(NH_4)₂SO₄ precipitation and subsequent DEAE-Affi-gel Blue chromatography in 20 mM-Tris/HCl buffer (pH 8.0) containing 28 mM-NaCl and 0.02% (w/v) NaN₃.

Inhibition of 5'-nucleotidase activity in various tissues from electric ray

Samples (1–2 g) of tissue (liver, heart, intestine and dorsal muscle) were excised and homogenized in 5 ml of 20 mM-Hepes buffer (pH 7.4). The homogenate was washed twice with 20 mM-Hepes by centrifugation [30 min at 35000 g (r_{av} 8.3 cm)] and resuspension. Membrane-bound enzymes were solubilized by dissolving the final pellet in 5 ml of 2% deoxycholate/5% Triton X-100 and stirring it for 1 h at room temperature. Unsolubilized material was separated by centrifugation [1 h at 35000 g (r_{av} 8.3 cm)], and the supernatant was chromatographed on a concanavalin A-Sepharose column (0.5 ml). Material bound to the column was eluted with 0.4 M- α -methyl mannoside and used for inhibition studies. For standardization, the extracts of the different tissues were diluted to the same activity of 5'-nucleotidase (500 nmol of P_i /h per ml). Portions (100 μl) of these extracts were preincubated for 0.5 h with different amounts of diluted antiserum (corresponding to 0–250 nl of the original antiserum). Residual 5'-nucleotidase activity was assayed as described above (Lanzetta *et al.*, 1979). For determination of non-specific phosphatase activity in tissues and purified fractions, β -glycerophosphate (0.5 mM) was used instead of AMP as a substrate.

Immunohistochemical techniques

Immunohistochemistry was performed essentially as described by Volknandt & Zimmermann (1986). Dilution of the first antiserum was 1:50 in TBSA (0.15 M-NaCl/10 mM-Tris/HCl, pH 7.4, containing 1% bovine serum albumin). As a control, preimmune serum (1:50) was applied in the same manner as anti-5'-nucleotidase antiserum.

RESULTS

Isolation of 5'-nucleotidase

The isolation procedure involves the preparation of a crude plasma-membrane fraction and a differential-solubilization step by Triton X-100 and deoxycholate. Triton X-100 (1%) solubilizes more than 70% of the proteins, but only a small amount of 5'-nucleotidase activity. A complete solubilization of 5'-nucleotidase is achieved by a combination of 5% Triton X-100 and 2% deoxycholate. The subsequent concanavalin A-Sepharose chromatography was performed at room temperature, and the enzyme was eluted with α -methyl mannoside. It was essential to store the eluted enzyme at -18°C at least overnight before applying it to the AMP-Sepharose. Omitting this step leads to a tight binding of the protein to the AMP-Sepharose and a very poor recovery. Other affinity media, such as ADP-Sepharose and NAD-Sepharose, can be used with the same efficiency (results not shown). Elution with 5 mM-AMP results in a pure enzyme fraction, which was dialysed and stored at -18°C . A 1500-fold purification of the enzyme was achieved from the crude pellet fraction (P_1) to the fraction eluted from the AMP-Sepharose column (Table 1).

Table 1. Purification of 5'-nucleotidase from *Torpedo* electric organ

The Table lists the steps of 5'-nucleotidase isolation. A unit represents 1 μ mol of AMP hydrolysed/min at 25 °C. Results are for material derived from 100 g of electric organ.

Fraction	Total activity (units)	Total protein (mg)	Specific activity (units/mg of protein)	Recovery (%)	Purification (fold)
P ₁ (crude membranes)	2.665	131	0.020	100	1
P ₂ (Triton X-100 extract)	2.160	45.9	0.047	81	2.3
S ₃ (Triton X-100/deoxycholate extract)	2.191	9.0	0.243	82.2	11.9
Concanavalin A-Sepharose chromatography	1.248	1.43	0.872	46.8	42.9
AMP-Sepharose chromatography	1.156	0.037	31.260	43.4	1539

Fig. 1. Analysis of 5'-nucleotidase by SDS/polyacrylamide-gel electrophoresis and immunoblotting

Lanes: (a) Standard proteins (myosin, M_r 205000; β -galactosidase, 116000; phosphorylase *b*, 97000; bovine serum albumin, 66000; ovalbumin, 47000; carbonic anhydrase, 29000); (b) purified 5'-nucleotidase after SDS/polyacrylamide-gel electrophoresis and silver staining; (c) purified 5'-nucleotidase after transfer to nitrocellulose and staining with concanavalin A-peroxidase; (d) 5'-nucleotidase after transfer to nitrocellulose and staining with anti-5'-nucleotidase antiserum (dilution 1:50); (e) synaptosome fraction derived from electric organ after transfer to nitrocellulose and staining with anti-5'-nucleotidase antiserum (dilution 1:50).

Molecular characterization of 5'-nucleotidase

The purified enzyme reveals a single band after SDS/polyacrylamide-gel electrophoresis followed by silver staining (Fig. 1). The apparent M_r was 62000. When the native enzyme was applied to a Sephacryl 200 column equilibrated with 0.6% deoxycholate, 5'-

nucleotidase activity was eluted at a volume corresponding to a M_r of 131000. However, this value represents the M_r of the protein-detergent complex and not that of the pure protein. If Triton X-100, which forms very large micelles, is used, the enzyme activity is eluted with the void volume.

The hydrolysis of nucleoside monophosphates and other phosphate esters is shown in Table 2. Highest activity is obtained with AMP and CMP; UMP, GMP and IMP are hydrolysed to a lower extent. There is only a small activity towards deoxyribonucleoside monophosphates, as shown for dAMP and dGMP. No activity of non-specific phosphatases is present in the preparation, although minor amounts of ADP and cyclic AMP are hydrolysed by the enzyme. This may be due to contamination of these substances by AMP.

Activity of purified 5'-nucleotidase is maximal in the neutral pH range (pH 7.0–7.5). The pH/activity curve is not modified by the addition of $MgCl_2$ (2 mM) to the incubation medium (results not shown).

Effects of bivalent metal ions

5'-Nucleotidase is active in the absence of exogenous bivalent metal ions (Fig. 2). It is slightly activated by Mg^{2+} , Ca^{2+} and Sr^{2+} in the millimolar range. The maximal activation observed at a concentration of 2 mM- Ca^{2+} was 40%. Cu^{2+} and Mn^{2+} had no effect, whereas Hg^{2+} decreased enzyme activity by 50%. Almost complete inhibition was obtained after addition of 1 mM- Zn^{2+} or $-Pb^{2+}$.

Inhibitors of 5'-nucleotides

The substrate analogue adenosine 5'-(α,β -methylene]-diphosphate inhibits enzyme activity very effectively ($K_{i, app.}$ 0.85 μ M) (Table 2). Inhibition by ATP was less pronounced than by ADP. Lineweaver-Burk plots reveal a competitive inhibition pattern of all three inhibitors. Concanavalin A inhibits enzyme activity in a non-competitive manner, without affecting the K_m for AMP.

The mannose-specific lectins concanavalin A and *Lens culinaris* lectin (80 ng of purified enzyme plus 100 μ g of lectin/ml) decreased enzyme activity to 16.1 and 81.5% of control activity respectively ($n = 2$). Lectins specific for *N*-acetyl-D-glucosamine (wheat-germ agglutinin), α -L-fucose (Lotus lectin), β -D-galactosyl-(1-3)-*N*-acetyl-D-galactosamine (peanut lectin) and *N*-acetyl-D-

Table 2. Properties of 5'-nucleotidase purified from *Torpedo* electric organ

Substrate specificity (all substances 0.5 mM) was analysed by measuring the release of P_i by the method of Lanzetta *et al.* (1979). The 100% value corresponds to the V_{max} value of AMP. For determination of kinetic constants the radiochemical assay of Gentry & Olson (1975) was used. K_i values were calculated from Dixon plots.

Substrate specificity	AMP (100%), CMP (93%), UMP (80%), IMP (63%), GMP (52%), dAMP (14%), dGMP (7%), ADP (4.5%), 3'-AMP (2.4%), 3',5'-cyclic AMP (0.7%), ATP, β -glycerophosphate, phosphoenolpyruvate, <i>p</i> -nitrophenyl phosphate (not detectable)
K_m (AMP)	38 μ M, V_{max} = 31 μ mol/min per mg of enzyme
Competitive inhibitors	Adenosine 5'-[$\alpha\beta$ -methylene]diphosphate, K_i = 0.85 μ M ADP, K_i = 22 μ M ATP, K_i = 48 μ M
Non-competitive inhibitor:	Concanavalin A, K_i = 0.17 μ M
M_r determined by SDS/polyacrylamide-gel electrophoresis	62000
M_r determined by chromatography in 0.6% deoxycholate	131000

Fig. 2. Effect of bivalent metal ions on the activity of 5'-nucleotidase

The purified enzyme was first dialysed against Tris/HCl buffer (50 mM, pH 7.5). Stock solutions of metal ions were prepared in the same buffer. Enzyme activity (0.5 mM-AMP) was determined by the radiochemical method of Gentry & Olson (1975). Metal ions were used as chloride salts, except for $CuSO_4$, $ZnSO_4$ and lead acetate. The 100% value corresponds to an activity of 31 units/mg of protein. Results are means of triplicate determinations in one experiment. $\circ$, Mg^{2+} ; $\square$, Ca^{2+} ; $\triangle$, Sr^{2+} ; $\blacktriangle$, Cu^{2+} ; $\blacksquare$, Mn^{2+} ; $\blacklozenge$, Hg^{2+} ; $\bullet$, Zn^{2+} ; $\diamond$, Pb^{2+} .

galactosamine (*Vicia villosa* lectin) are without effect. Binding of concanavalin A to 5'-nucleotidase can also be demonstrated by concanavalin A-peroxidase staining of 5'-nucleotidase after transfer of the enzyme to nitrocellulose (Fig. 1c).

Characterization of the antiserum directed against 5'-nucleotidase

The polyclonal antiserum raised against the native enzyme obtained after AMP-Sepharose chromatography

Fig. 3. Tissue specificity of 5'-nucleotidase as revealed by enzyme inhibition with monospecific antiserum

The 100% values represent total activity with AMP as substrate minus the activity of non-specific phosphatases assayed with β -glycerophosphate as a substrate. No inhibition was obtained with preimmune serum. Tissue: $\circ$, liver; $\triangle$, dorsal muscle; $\square$, heart; $\blacktriangle$, intestine; $\bullet$, electric organ.

inhibited the activity of the isolated enzyme by up to 100%. The antiserum directed against the denatured antigen reacted in immunofluorescence studies and in immunoblotting (results not shown), but failed to inhibit 5'-nucleotidase activity. Throughout this study we used the antiserum raised against the native antigen. Monospecificity of the polyclonal antiserum could be shown by an immunoblotting technique (Western blot) (Fig. 1). Purified antigen as well as a synaptosomal fraction were

Fig. 4. Immunocytochemical localization of 5'-nucleotidase in the electric organ

(a) Immunofluorescence with FITC-labelled second antibody; (b) corresponding phase-contrast image: d, dorsal side of electroplaque cells; i, interstitial space between electroplaque cells; v, ventral side of electroplaque cells with pattern of nerve-terminal ramifications; arrows: axons in interstitial space. Bar represents 10 μ m.

transferred to nitrocellulose after SDS/polyacrylamide-gel electrophoresis. The antiserum recognizes only a single band, with an M_r of 62000.

Tissue specificity of 5'-nucleotidase

The inhibitory effect of the antiserum raised against electric-organ 5'-nucleotidase was tested against crude enzyme fractions derived from muscle, heart, liver and intestine. Owing to the high activity of non-specific phosphatases in tissues other than electric organ, fractions had to be partially purified before testing the inhibitory effect of the anti-5'-nucleotidase antiserum. This was performed by extraction of tissues with deoxycholate and Triton X-100 followed by concanavalin A-Sepharose chromatography as described in the Experimental section. After this step, the percentage of non-specific phosphatase contributing to the total P_i -releasing activity was less than 10% for liver and heart. Dorsal muscle still contained 11% non-specific phosphatase activity, and in the intestine extract this amounted to as much as 45%. Taking into account these background values, the degree of inhibition obtained by the application of the anti-5'-nucleotidase antiserum could be calculated. Fig. 3 shows almost complete inhibition of 5'-nucleotidase activity in total electric organ and intestine extracts. The enzyme from heart and dorsal muscle could be inhibited by up to 70%. Even at high antiserum concentrations, activity of the liver 5'-nucleotidase is decreased by only 20%.

Tissue distribution of 5'-nucleotidase

By application of immunocytochemical methods, 5'-nucleotidase can be localized to the nerve-terminal

ramifications which cover the ventral surface of the electroplaque cell as a dense net-like structure (Fig. 4). There is also a faint but significant staining of the dorsal side of the electroplaque cell, which is free of nerve terminals and which carries numerous and extensive plasma-membrane infoldings (Whittaker & Zimmermann, 1976). Control sections incubated with preimmune serum reveal a faint and unspecific background staining of all parts of the tissue (results not shown).

DISCUSSION

5'-Nucleotidase isolated from the electric organ of *Torpedo marmorata* shares several consistent molecular properties with the same enzyme isolated from a wide variety of tissues and cells. It is a membrane glycoprotein, and enzyme activity can be inhibited (in a non-competitive manner) by the lectin concanavalin A and to a small extent by *Lens culinaris* lectin, which both are specific for D-glucose and D-mannose (Riordan & Slavik, 1974). Heterogeneity of the glycosidic moiety of the enzyme has been reported, and, in contrast with the enzyme derived from *Torpedo* electric organ, wheat-germ agglutinin may be inhibitory to 5'-nucleotidase in some tissues (Meflah *et al.*, 1984a).

The K_m value for AMP is in the low micromolar range, and generally AMP is the most effectively hydrolysed mononucleotide. However, in some cases pyrimidine-ribose nucleotides such as IMP and others have been reported to be hydrolysed in preference to AMP (Tanaka *et al.*, 1973; Van den Berghe *et al.*, 1977; Montero & Fes, 1982). Similarly ATP, ADP, and in particular adenosine 5'-[α,β -methylene]diphosphate, are effective inhibitors of

5'-nucleotidase (Burger & Lowenstein, 1970; Newby *et al.*, 1975; Naito & Lowenstein, 1981). In *Torpedo* inhibition is competitive for all three substances. 5'-Nucleotidase appears to be a metalloenzyme, and metal-ion chelators such as EDTA and EGTA inhibit enzyme activity. The nature of the endogenous cation is still uncertain (Harb *et al.*, 1984). The enzyme purified from *Torpedo* electric organ, like that in other tissues, is activated by the addition of Mg^{2+} , whereas Zn^{2+} is a very strong inhibitor (Tanaka *et al.*, 1973; Stefanovich *et al.*, 1976; Cammer *et al.*, 1980; Mallol & Bozal, 1983; Meflah *et al.*, 1984b).

The enzyme has been purified to homogeneity from a variety of tissue sources, and the apparent M_r obtained on SDS/polyacrylamide-gel electrophoresis is generally around 70000 (Fukui & Shichi, 1981; Naito & Lowenstein, 1981; Bailyes *et al.*, 1984; Dieckhoff *et al.*, 1985). With an apparent M_r of 62000 the *Torpedo* enzyme is somewhat smaller, but the M_r of this glycoprotein may vary to some extent with the experimental conditions used. Our observation that under native conditions and on gel filtration in the presence of deoxycholate the enzyme is eluted at an M_r of 131000 would be consistent with the notion that the enzyme occurs as a homodimer (Naito & Lowenstein, 1981; Montero & Fes, 1982; Harb *et al.*, 1983; Bailyes *et al.*, 1984; Dieckhoff *et al.*, 1985).

5'-Nucleotidase is considered to be a short-stalked integral membrane protein (Baron *et al.*, 1986). By using high concentrations of phosphatidyl inositol-specific phospholipase C from *Staphylococcus aureus*, part of the 5'-nucleotidase activity could be released from isolated hepatocytes (Shukla *et al.*, 1980), suggesting that phosphatidylinositol is involved in anchoring the enzyme in the membrane. Using this enzyme (gift from Dr. M. G. Low, Oklahoma Medical Research Foundation, Oklahoma City, OK, U.S.A.), we obtained less than 10% release of 5'-nucleotidase activity from electric-organ synaptosomal fractions under conditions which released 50% of acetylcholinesterase (E. J. M. Grondal & H. Zimmermann, unpublished work). If 5'-nucleotidase is anchored via phosphatidylinositol, it is far less acceptable to the phospholipase than is acetylcholinesterase.

The inability of the antiserum raised against electric-organ 5'-nucleotidase to inhibit enzyme activity completely in some other tissues suggests that tissue-specific variants of the enzyme exist in *Torpedo*. This is in accordance with the detection of tissue-specific variants in studies using antibodies against chicken gizzard and bovine brain 5'-nucleotidase (Dieckhoff *et al.*, 1985; Harb *et al.*, 1985).

The immunocytochemical localization of the enzyme in the electric organ suggests that it is present along the surface of nerve terminals throughout the region of synaptic contact and that it would be in a location to participate in the complete hydrolysis of extracellular ATP at the synapse, as hypothesized previously (Zimmermann *et al.*, 1979). The terminal ramifications of the axons inside the electric organ are surrounded by a very thin lamella of Schwann-cell processes to the site of synaptic contact (Whittaker & Zimmermann, 1976). We cannot decide from the present evidence whether the enzyme is associated with this fine lamella or with the axonal plasma membrane itself.

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