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Nucleotidase activities in membrane preparations of nervous ganglia and digestive gland of the snail *Helix aspersa*

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Abstract

Nucleotide-metabolizing enzymes play an important role in the regulation of nucleotide levels. In the present report, we demonstrated an enzyme activity with different kinetic properties in membrane preparations of the nervous ganglia and digestive gland from *Helix aspersa*. ATPase and ADPase activities were dependent on Ca^{2+} and Mg^{2+} with pH optima approximately 7.2 and between 6.0 and 8.0 in digestive gland and nervous ganglia, respectively. The enzyme activities present in membrane preparations of these tissues preferentially hydrolyzed triphosphate nucleotides. In nervous ganglia, the enzyme was insensitive to the classical ATPases inhibitors. In contrast, in digestive gland, *N*-ethylmaleimide (NEM) produced 45% inhibition of Ca^{2+}-ATP hydrolysis. Sodium azide, at 100 μM and 20 mM, inhibited Mg^{2+}-ATP hydrolysis by 36% and 55% in digestive gland, respectively. The presence of nucleotide-metabolizing enzymes in these tissues may be important for the modulation of nucleotide and nucleoside levels, controlling their actions on specific purinoceptors in these species.

Keywords: Adenosine; ATPase; Digestive gland; Mollusks; Nervous ganglia; Nucleotidases; *Helix aspersa*; ATP

1. Introduction

ATP has been shown to be stored in nerve terminals and released with several neurotransmitters, including acetylcholine, noradrenaline and serotonin (Di Iorio et al., 1998; Rathbone et al., 1999). Extracellular ATP has been established as a signaling molecule, which mediates its actions through two subclasses of P2 purinoceptors: metabotropic P2Y receptors and ionotropic P2X receptors (Cunha, 2001; Bodin and Burnstock, 2001; Khakh et al., 2001). Signalling actions of nucleotides require effective mechanisms for inactivation (Zimmermann, 1996, 2001). After its release, ATP can be hydrolyzed into the nucleoside adenosine, an important neuromodulator, by a group of enzymes located on the cell surface called ecto-nucleotidases. This family of enzymes includes an ATP diphosphohydrolase (apyrase, NTPDase 1, EC 3.6.1.5), ecto-ATPase (NTPDase 2, EC 3.6.1.3) and ecto-5′-nucleotidase (EC 3.1.3.5) (Sarkis et al., 1995; Zimmermann, 1996, 2001). These enzymes may participate in the control of nucleotide and nucleoside levels in the synaptic cleft and, consequently, in the control of purinergic

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neurotransmission (Zimmermann et al., 1998; Rathbone et al., 1999; Sebastião et al., 1999).

The effects of purine nucleotides and nucleosides have been widely studied in several classes of vertebrates (Ralevic and Burnstock, 1998); however, there have been few studies conducted on adenosine and ATP receptor-mediated actions in mollusks (Hoyle and Greenberg, 1988; Knight et al., 1992a,b; Vignola et al., 1995; Michaelidis et al., 2002). Recently, we characterized an ATPase from the nervous ganglia of *Phyllocaulis soleiformis*, which presented a peculiar and different kinetic behavior when compared to other ATPases described in vertebrates (Da Silva et al., 2002).

The common garden snail *Helix aspersa* (Müller, 1774) (Mollusca, Gastropoda, Helicidae) is a terrestrial mollusk. Several researchers have used this snail as an experimental model for biochemical, physiological, cytological, cytochemical and toxicological studies (Janahmadi et al., 1999). Considering that the molluscan digestive gland and nervous ganglia perform multiple functions in the physiology of the animal, the purpose of this study is to characterize the degradation of ATP and ADP in both tissues of *H. aspersa*, starting with an enzymatic characterization.

2. Materials and methods

2.1. Experimental model

Adult *H. aspersa* snails were collected all year long from gardens (pesticide free) of metropolitan region of Porto Alegre, RS, Brazil. Animals were maintained in plastic boxes ($68\times60\times22$ cm) at 25 ± 5 °C, in a photoperiod of 12 h light/12 h dark for at least 7 days before experiments. Snails were fed with lettuce (*Latuca sativa*) ad libitum.

2.2. Membrane preparation of the nervous ganglia and digestive gland

In *H. aspersa*, the nervous system is composed of ganglia and their connectives fused into a circumesophageal ring (South, 1992). The ring was isolated from the anterior region of the animal using a single-sided razor blade. Under the stereomicroscope, the nervous ring was separated and added to a solution of NaCl (0.65%) containing a protease inhibitor (0.1 mM PMSF), at 4 °C. To isolate the digestive gland, the shell was removed. The membrane preparations were made according to Barnes et al. (1993). Briefly, the ring of nervous ganglia and digestive gland were homogenized in 25 vol. (w/v) and 5 vol. (w/v), respectively, in a solution of NaCl (0.65%) containing a protease inhibitor (0.1 mM PMSF). The homogenates were centrifuged at $1000\times g$ for 10 min, the pellet discarded, and the supernatant centrifuged for 20 min at $40\,000\times g$. The pellets were frozen in liquid nitrogen for 10 s, thawed, resuspended twice and centrifuged for 20 min at $40\,000\times g$. The membranes were prepared fresh daily and maintained at 4 °C throughout the preparation and experiment.

2.3. Enzyme assays

Enzyme activity was assayed in standard reaction medium containing 50 mM Tris–HCl, pH 7.2, 5 mM $CaCl_2$ or $MgCl_2$ in a final vol. of 200 μl. Membranes of nervous ganglia and digestive gland of *H. aspersa* (5–10 μg and 2.5–5 μg protein, respectively) were added to the reaction medium and pre-incubated for 10 min at 30 °C. The reaction was initiated by the addition of substrate (ATP or other, as indicated) at a final concentration of 1 mM, incubated for 20 min at 30 °C and stopped by addition of 200 μl 10% trichloroacetic acid (TCA). The samples were chilled on ice for 10 min, and the inorganic phosphate (P_i) released was measured according to Chan et al. (1986). Incubation times and protein concentrations were chosen in order to ensure the linearity of the reactions. Controls with the addition of the enzyme preparation after mixing with TCA were used to correct for non-enzymatic hydrolysis of substrates. To determine the optimum pH for the enzyme, Ca^{2+}-ATP and Ca^{2+}-ADP hydrolysis in membrane preparations from nervous ganglia and Ca^{2+}-ATP and Mg^{2+}-ATP in membrane preparations from digestive gland were used as substrates in a medium consisting of 50 mM Tris–histidine at different pH values (pH 6.0, 7.0, 7.2, 8.0 and 9.0).

To determine the kinetic constants for the enzymes, Ca^{2+}-ATP and Ca^{2+}-ADP hydrolysis in membrane preparations of nervous ganglia and Ca^{2+}-ATP and Mg^{2+}-ATP hydrolysis in digestive gland from *H. aspersa* were incubated at different concentrations ranging from 150 to 1000 μM for each substrate. K_m and V_{max} values for the ATPase/ADPase activities from nervous ganglia and digestive gland of *H. aspersa* were estimated from the Lineweaver–Burk plot with five different enzyme preparations (Lineweaver and Burk,

1934). To evaluate other possible enzyme activities involved in nucleotide hydrolysis in nervous ganglia and digestive gland, the effect of various classical ATPase inhibitors was tested in both tissues. Specific activity was expressed as nmol P_i min^{-1} mg^{-1} protein. Each condition tested in enzyme assays was run in triplicate.

2.4. Protein determination

Protein was determined by the Coomassie Blue method, using bovine serum albumin as a standard (Bradford, 1976).

2.5. Statistical analysis

Data were expressed as means ± S.D. and were analyzed using Student's *t*-test or one-way analysis of variance (ANOVA), followed by Duncan's test as a post hoc test. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Time course and protein concentration

We determined the time course for ATP and ADP hydrolysis from membrane preparations of nervous ganglia and digestive gland of *H. aspersa* at several incubation times (5, 10, 20 and 30 min). The results indicated that ATP or ADP hydrolysis was linear up to 30 min; however, in order to ensure that the incubation time was within the linear portion of the reaction, we chose 20 min as the assay time for both fractions in subsequent experiments (data not shown). In relation to protein concentration, we tested the effect of different protein concentrations (1, 2.5, 5, 10 and 20 μg) on ATP and ADP hydrolysis in membrane preparations of nervous ganglia and digestive gland. The results demonstrated that ATP and ADP hydrolysis were linear up to 20 μg protein. However, in subsequent experiments, we used 5–10 μg protein for ATP and ADP hydrolysis in nervous ganglia and 2.5–5 μg protein for ATP hydrolysis in digestive gland (data not shown).

Although ADP hydrolysis is higher in digestive gland than in nervous ganglia (Table 1), it was not linear with the increase of incubation time and protein concentration. Therefore, only ATP hydrolysis was evaluated in membrane preparations of digestive gland.

Table 1
Substrate specificity of Ca^{2+}-ATPase in nervous ganglia and Ca^{2+}-ATPase and Mg^{2+}-ATPase activities in digestive gland of *H. aspersa*

Substrate	% Activity		
	Nervous ganglia Calcium	Digestive gland	
		Calcium	Magnesium
ATP	100 ± 10	100 ± 16	100 ± 11
GTP	94 ± 18	74 ± 13	83 ± 10
UTP	93 ± 9	42 ± 8	48 ± 10
CTP	62 ± 4	16 ± 2	35 ± 6
ADP	6 ± 1	27 ± 3	14 ± 3
GDP	6 ± 1	22 ± 4	7 ± 2
UDP	5 ± 1	25 ± 2	13 ± 2
AMP	ND	ND	4 ± 0.2
PP_i	0.3 ± 0.1	4 ± 1	28 ± 5

Results are expressed as mean ± S.D. of at least five experiments. All substrates were used at 1.0 mM with 5 mM Ca^{2+} or 5 mM Mg^{2+}. Control Ca^{2+}-ATPase was 1086.8 ± 96.9 nmol P_i min^{-1} mg^{-1} protein in nervous ganglia, and Ca^{2+}-ATPase and Mg^{2+}-ATPase activities were 161.3 ± 26.4 nmol P_i min^{-1} mg^{-1} protein and 268 ± 29 nmol P_i min^{-1} mg^{-1} protein, respectively, in digestive gland. ND, not detectable.

3.2. Cation and pH dependence

To further optimize assay conditions, the effect of different Ca^{2+} and Mg^{2+} (chloride salts) concentrations on ATP and ADP hydrolysis was investigated (Fig. 1). In both tissues the results showed an increase in enzyme activities in the presence of different concentrations of these cations. A significant activation of ATP hydrolysis was observed in the presence of 5 mM Mg^{2+} plus 5 mM EDTA in membrane preparations of nervous ganglia, but this effect was not observed with ADP hydrolysis under the same conditions (Fig. 1a,b). In digestive gland, observed ATP hydrolysis with a concentration of 1–10 mM Mg^{2+} is higher than in the presence of Ca^{2+} (Fig. 1c). In subsequent experiments, a concentration of 5 mM was used for both cations. In nervous ganglia, pH-dependent curves using either ATP or ADP as substrate showed the highest activity at pH 6.0–7.2 and pH 6.0–8.0, respectively, (Fig. 2a,b). The maximal rate of ATP hydrolysis in digestive gland was observed at pH 7.2–9.0 in the presence of 5 mM Ca^{2+}, and pH 7.2 with 5 mM Mg^{2+} (Fig. 2c, d).

3.3. Kinetic constants

Ca^{2+}-ATP and Ca^{2+}-ADP hydrolysis in membrane preparations of nervous ganglia and

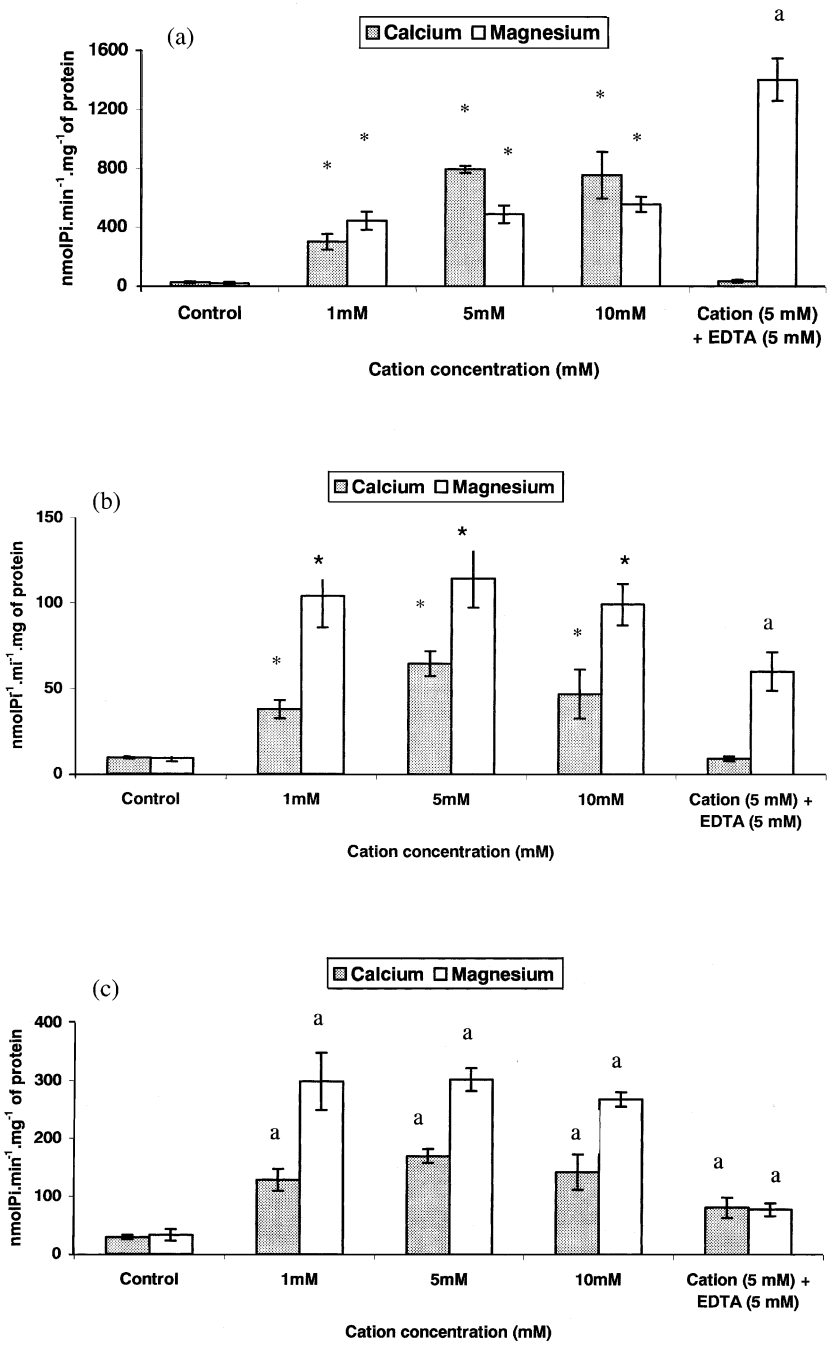

Fig. 1. Effect of different concentrations of $CaCl_2/MgCl_2$-ATP (a) and $CaCl_2/MgCl_2$-ADP (b) in membrane preparations of nervous ganglia and $CaCl_2/MgCl_2$-ATP (c) in digestive gland from *H. aspersa*. Control group was incubated without addition of cation. Enzyme assay conditions are described in Section 2 Data represent means ± S.D. of five different experiments, each in triplicate. * indicates difference when compared to the control and 5 mM calcium + EDTA group. [a]indicates difference when compared to the control group. Data were analyzed statistically by one-way (ANOVA) followed by Duncan's test as a post hoc test, $P < 0.05$.

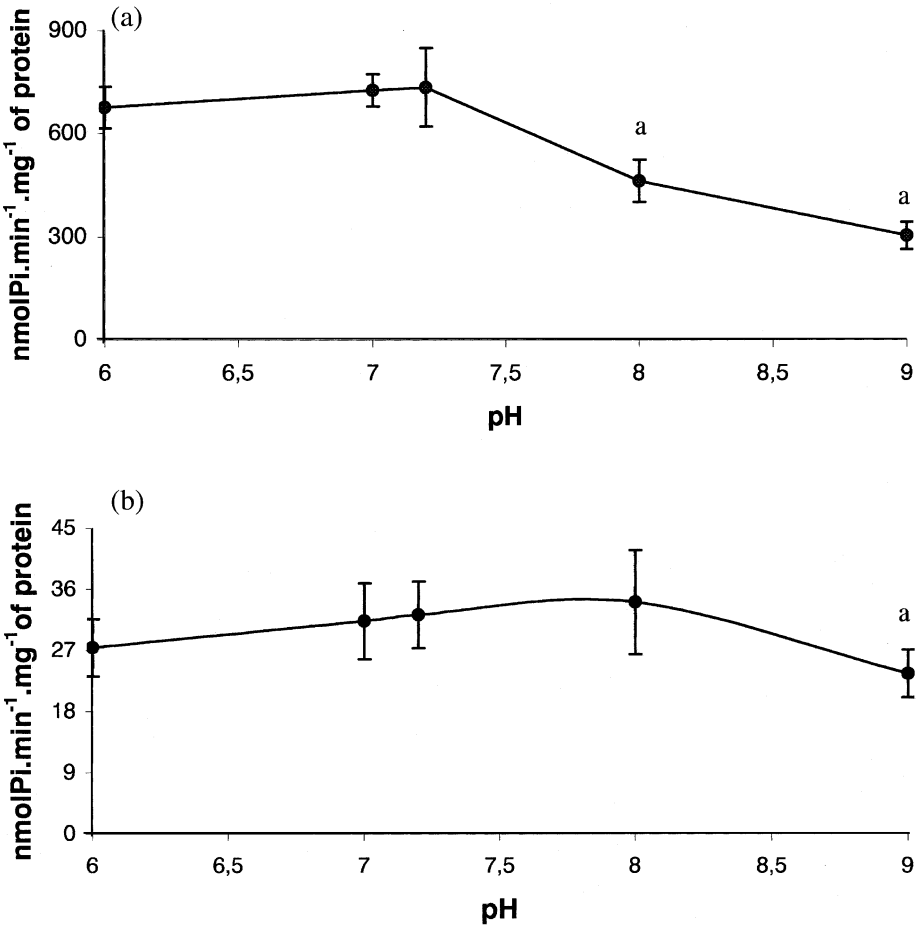

Fig. 2. pH dependence of Ca^{2+}-ATP (a) and Ca^{2+}-ADP (b) hydrolysis in membrane preparations of nervous ganglia; Ca^{2+}-ATP (c) and Mg^{2+}-ATP (d) hydrolysis in digestive gland from *H. aspersa.* Enzyme activity was determined as described in Section 2 using the following buffers: 50 mM Tris–histidine, pH 6.0, 7.0, 7.2, 8.0 and 9.0. Enzyme assay conditions are described in Section 2. Data represent means ± S.D. of five different experiments, each in triplicate. [a]indicates difference when compared to the values obtained at pH 7.2. Data were analyzed statistically by one-way (ANOVA) followed by Duncan's test as a post hoc test, $P < 0.05$.

Ca^{2+}-ATP and Mg^{2+}-ATP hydrolysis in membrane preparations of digestive gland from *H. aspersa* were determined at different concentrations ranging from 150 to 1000 μM for each substrate (Fig. 3). The apparent K_m for Ca^{2+}-ATP and Ca^{2+}-ADP from nervous ganglia was 588.8 ± 74.5 μM and 726.3 ± 117.07 μM, respectively. V_{max} values for Ca^{2+}-ATP and Ca^{2+}-ADP were 3538.9 ± 827.7 nmol P_i min^{-1} mg^{-1} protein and 268.5 ± 57.8 nmol P_i min^{-1} mg^{-1} protein, respectively. In digestive gland, the K_m for Ca^{2+}-ATP and Mg^{2+}-ATP was 465 ± 86 μM and 172.6 ± 8.4 μM, respectively. V_{max} values in digestive gland for Ca^{2+}-ATP and Mg^{2+}-ATP were 407.5 ± 89 nmol P_i min^{-1} mg^{-1} protein and 244 ± 8.5 nmol P_i min^{-1} mg^{-1} protein, respectively.

3.4. Substrate specificity and effect of inhibitors

The ability to hydrolyze other mono, di and triphosphate nucleosides was also investigated (Table 1). The enzyme activities present in the membrane preparations of nervous ganglia were able to preferentially hydrolyze triphosphate nucleotides. Triphosphate nucleotides were also hydrolyzed at a higher rate in membrane preparations from digestive gland, while the hydrolysis of diphosphate nucleotides was increased in this tissue when compared to nervous ganglia. PP_i pre-

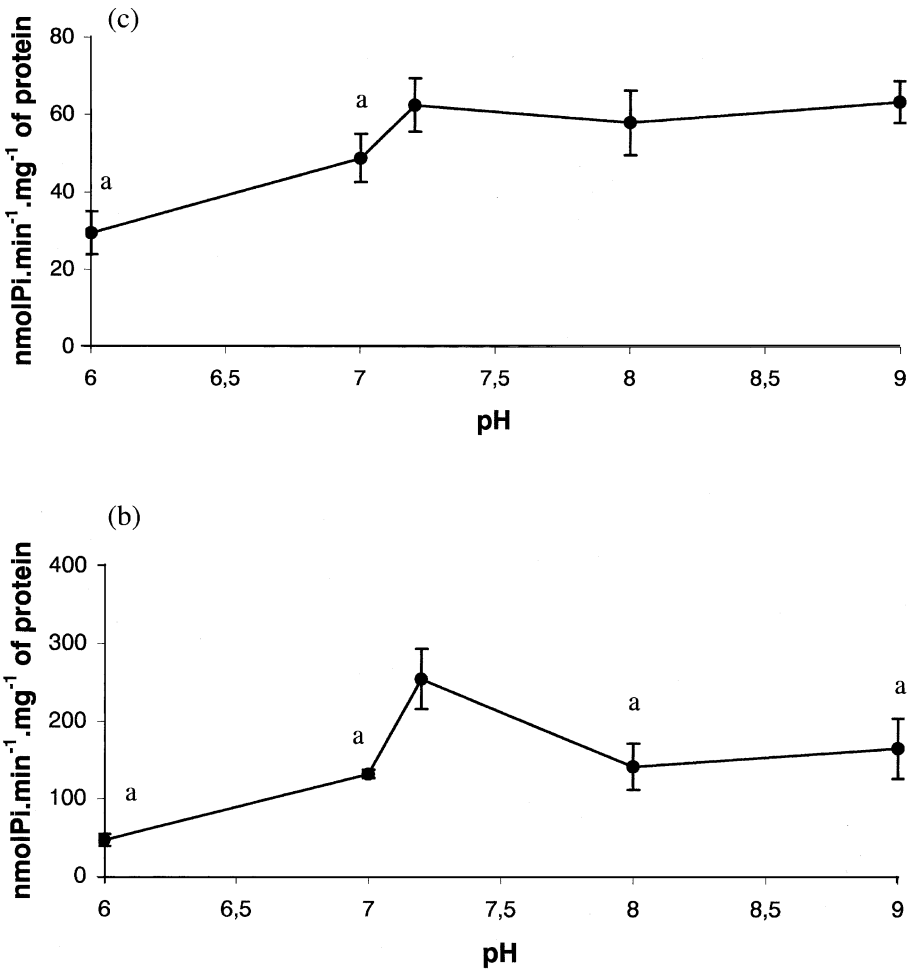

Fig. 2 (Continued).

sented higher hydrolysis in the presence of magnesium in this tissue.

The following ATPase and alkaline phosphatase inhibitors had no effect upon ATP and ADP hydrolysis in membrane preparations of nervous ganglia from *H. aspersa* (Table 2): (a) ouabain and orthovanadate, used as Na^+, K^+-ATPase inhibitors (Besch et al., 1976; Cantley et al., 1978); (b) *N*-ethylmaleimide (NEM), a specific SH-group inhibitor; (c) sodium azide (<5 mM), a mitochondrial ATPase inhibitor; (d) sodium azide (>5 mM), an NTPDase 1 (ATP diphosphohydrolase) inhibitor; and (e) levamisole, a specific inhibitor of alkaline phosphatase. However, in digestive gland, *N*-ethylmaleimide (10 mM) and sodium azide (100 μM and 20 mM) demonstrated significant inhibition of Ca^{2+}-ATP (45%) and Mg^{2+}-ATP (36 and 55%) hydrolysis, respectively, (Table 2).

4. Discussion

The comparison of enzyme activities in membrane preparations of nervous ganglia and digestive gland showed different kinetic properties. The results demonstrated ATP and ADP hydrolysis in membrane preparations of nervous ganglia and digestive gland. Nervous ganglia showed maximal ATP and ADP hydrolysis in the presence of 5–10 mM calcium and 1–10 mM magnesium, respectively, (Fig. 1a,b). However, $CaCl_2$ was utilized, because it is an important physiological activator. Furthermore, $CaCl_2$ was used in the experiments of this study in order to exclude the significant

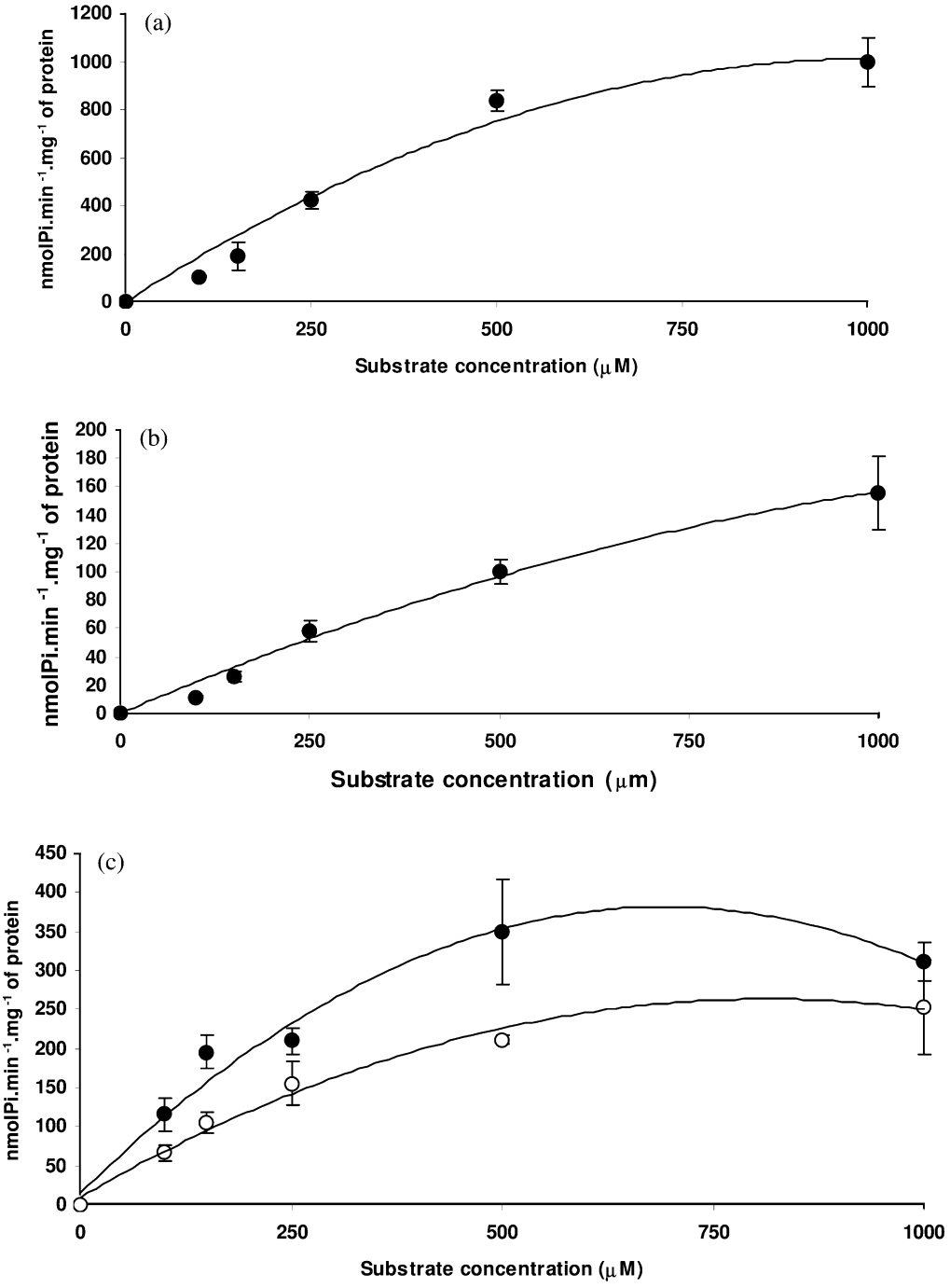

Fig. 3. Effect of different concentrations of substrate (150–1000 μM) on nucleotide hydrolysis in nervous ganglia and digestive gland of *H. aspersa*. (a) Ca^{2+}-ATP hydrolysis in nervous ganglia, (b) Ca^{2+}-ADP hydrolysis in nervous ganglia, (c) Mg^{2+}-ATP (●) and Mg^{2+}-ADP (○) hydrolysis in digestive gland. All experiments used fixed Ca^{2+} or Mg^{2+} at 5 mM with variable concentrations of nucleotides. Data represent means ± S.D. of five different experiments, each in triplicate.

Table 2
Effects of inhibitors on ATP and ADP hydrolysis in nervous ganglia and in digestive gland of *H. aspersa*

Compounds	Concentration	% of control enzyme activity			
		Nervous ganglia		Digestive gland	
		Ca^{2+}-ATP	Ca^{2+}-ADP	Ca^{2+}-ATP	Mg^{2+}-ATP
Sodium vanadate	0.1 mM	91±19	116±21	100±21	84±14
Levamisole	1 mM	84±9	134±15	111±4	102±22
N-ethylmaleimide (NEM)	10 mM	85±18	100±11	55±9*	77±10
Ouabain	1 mM	113±19	123±15	107±7	87±13
Oligomycin	2.0 μg/ml	131±35	132±22	114±22	91±16
Sodium azide	100 μm	124±21	92±11	121±22	64±7*
	20 mM	87±14	94±15	95±18	45±4*

Control Ca^{2+}-ATPase and Ca^{2+}-ADPase activities in nervous ganglia and Ca^{2+}-ATPase and Mg^{2+}-ATPase activities in digestive gland were 900.8±131.7 nmol P_i min^{-1} mg^{-1} protein, 58.7±11.4 nmol P_i min^{-1} mg^{-1} protein, 82.6±13.6 nmol P_i min^{-1} mg^{-1} protein and 148.2±29.5 nmol P_i min^{-1} mg^{-1} protein, respectively. Results are expressed as percentages of control activity (100%). Data represent means±S.D. of five different experiments, each in triplicate. Data were analyzed statistically by one-way analysis of variance or by paired Student's *t*-test. *Values significantly different from control values (100%), ($P<0.05$).

interference of Mg^{2+}-dependent enzymes, such as Na^+, K^+-ATPase and adenylate kinase. The concentration used in the incubation medium (5 mM $CaCl_2$) is similar to the Ca^{2+} concentration found in the hemolymph of *Ariolimax columbianus* (6.6 mM), indicating that the medium used in our experiments reflects physiological conditions for Ca^{2+} concentration (Deyrup-Olsen et al., 1983). These results agree with those of the literature, which have identified a Ca^{2+}-activated ATPase in the nervous ganglia of a terrestrial slug (*P. soleiformis*; Da Silva et al., 2002). In relation to the digestive gland, Mg^{2+} was a better activator of ATP hydrolysis than Ca^{2+} in *H. aspersa*. Significant activation of ATP hydrolysis was observed in the presence of Mg^{2+} plus 5 mM EDTA in nervous ganglia, but this effect was not observed with ADP hydrolysis under the same conditions. This result suggests that EDTA could be exerting different effects on ATP and ADP hydrolysis in nervous ganglia.

The maximal rates of nucleotide hydrolysis in nervous ganglia were observed at pH 7.0–7.2 and 7.0–8.0 (Fig. 2a,b) and pH 7.2 in the digestive gland (Fig. 2c,d). These results agree with those from previous studies, which have shown a range similar to that cited for the hemolymph of some slugs, such as that of *Deroceras reticulatum*, with a pH of 7.72 (South, 1992).

Classical inhibitors of various intracellular ATPases, such as P-type, V-type and alkaline phosphatases, did not inhibit ATP and ADP hydrolysis in membrane preparations of nervous ganglia (Table 2). The concentrations of sodium azide required to inhibit mitochondrial ATPase and NTPDase 1 (ATP diphosphohydrolase, apyrase, EC 3.6.1.5) are in the 40–100 μM and 10–20 mM ranges, respectively, and no effect was observed on ATP and ADP hydrolysis at these concentrations in nervous ganglia (Plesner, 1995). The lack of inhibitory effect of classical ATPases and phosphatase alkaline inhibitors is similar to the insensitivity observed in ATP hydrolysis of the nervous ganglia from the slug *P. soleiformis* (Da Silva et al., 2002). In contrast, different results were observed in the membrane preparations of digestive gland of *H. aspersa*. In the presence of Ca^{2+}, *N*-ethylmaleimide (NEM) demonstrated significant inhibition, suggesting the presence of SH-groups in the enzyme (Table 2).

The molluscan digestive gland performs multiple functions of energy storage, metabolic transformation and enzyme manufacture (Flari and Charrier, 1992; Giard et al., 1995). Sodium azide, at low (100 μM) and high (20 mM) concentrations, decreased ATPase activity (Table 2). Azide is a well known inhibitor of mitochondrial ATPase with an I_{50} of ≈0.04 mM (Pullman et al., 1960), inhibiting both F_0F_1 and soluble F_1. The mechanism by which azide inhibits F_1 is not completely understood, although increasing evidence suggests that azide acts by stabilizing the inactive form of the enzyme (Vasilyeva et al., 1982; Hyndman et al., 1994). Furthermore, the inhibition observed in the presence of sodium azide (20 mM) suggests the possible participation of NTPDase activity in this tissue.

The ability to hydrolyze different di- and triphosphate nucleotides is a characteristic of enzymes of the ecto-nucleoside triphosphate

diphosphohydrolase family (E-NTPDase family). Table 1 shows that all purine and pyrimidine nucleotides tested were hydrolyzed by membrane preparations of nervous ganglia and digestive gland from *H. aspersa*. There is evidence that the differences in substrate preference between individual members of the E-NTPDase family may result from small differences in protein structure that affect substrate binding. Heine et al. (1999) provide a direct comparison of two members of E-NTPDase family named NTPDase 1 (ecto-apyrase) and NTPDase 2 (ecto-ATPase). The two enzymes differ mainly in their capacity to hydrolyze nucleoside-5′-diphosphates and share a broad substrate specificity towards purine and pyrimidine nucleotides. They differ in the sensitivity to known inhibitors of P2 receptors and can be differentiated by sequence-specific antibodies. In relation to the digestive gland, the triphosphate nucleotides were also hydrolyzed at a higher rate, and the hydrolysis of diphosphate nucleotides was increased in this tissue when compared to nervous ganglia. However, results obtained from our laboratory showed the presence of an ATPase unable to hydrolyze nucleotide 5′-diphosphates in *P. soleiformis* nervous ganglia (Da Silva et al., 2002).

Following a greater understanding of the importance of purinergic neurotransmission in vertebrate species (Burnstock, 1972; Baer and Drummond, 1979; Stone, 1981; Daly et al., 1983), the actions of purine compounds in invertebrate species have been investigated. In *H. aspersa* and slug *Arion ater*, effects of adenine nucleosides and nucleotides on the isolated heart were evaluated (Knight et al., 1992a). These authors demonstrated that adenosine, AMP, ADP and ATP (above 100 μM) produced either an excitation or an inhibition in the isolated heart from the snail *H. aspersa* and the slug *A. ater*, suggesting that the receptors activated by purine compounds in these two molluscan hearts are not comparable to the vertebrate purinoceptors (Burnstock, 1978; Ralevic and Burnstock, 1998). The administration of AMP, ADP and ATP in the snail *H. aspersa* produced concentration-dependent contractions in rectum and esophagus, suggesting that purinoceptors are important for these responses in mollusks (Knight et al., 1992b). Hoyle and Greenberg (1988) analyzed species belonging to several different invertebrate phyla and observed that the effects of the agonists to purinoceptors were extraordinarily varied, demonstrating some similarity with the effects observed in vertebrates. However, classical antagonists of purinoceptors were ineffective in the invertebrate tissues, indicating that the purinoceptors from invertebrates are distinct from those of vertebrates. In mollusks, the responses of purinoceptors differ due to variation within taxons from subspecies to subclass (Painter and Greenberg, 1982). In addition, the experimental conditions (Twarog, 1967), season (Prosser, 1940; Nistratova, 1981) and age (Chapman et al., 1984) have all been shown to influence the sensitivity and pharmacological response of isolated molluscan muscles and muscular organs (Hoyle and Greenberg, 1988).

It has been suggested that ATP is a primitive neurotransmitter since it is well represented in lower vertebrates (Burnstock, 1972, 1976). For this reason, the presence of enzymes able to hydrolyze nucleotides may be an important mechanism required to control the inactivation of these purinoceptors, by removal of ATP (agonist of P2X and P2Y receptors) and, consequently, production of adenosine (agonist of P1 receptors).

In the present study, the results demonstrate enzyme activities in membrane preparations of nervous ganglia and digestive gland of *H. aspersa* with different kinetic characteristics than those previously described (Plesner, 1995; Sarkis et al., 1995; Da Silva et al., 2002). The analysis of these enzyme activities in *H. aspersa* will contribute to the understanding of the physiological significance in regulating the extracellular and intracellular nucleotide levels in the snail.

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