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Ecto-nucleotidase of cultured rat superior cervical ganglia: dipyridamole is a novel inhibitor

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Abstract

Based on studies of agonist potencies on intact rat superior cervical ganglia, it has been suggested that this ganglion possesses distinct receptors for purine and pyrimidine nucleotides. However, the potency of an agonist is dependent upon whether it is susceptible to extracellular metabolism by the tissue. The aim of this investigation was to study the metabolism of uridine or adenosine nucleotides and nucleosides and the effects of dipyridamole and an ecto-ATPase inhibitor ARL 67156 (6-*N*,*N*-diethyl-D-beta-gamma-dibromomethylene-ATP) on their metabolism. Adenosine- and uridine-5′-triphosphates (ATP and UTP) were catabolised by cultured rat superior cervical ganglia, to their di- and monophosphates. Both ATP and UTP breakdown was significantly inhibited by dipyridamole (10 μM), whereas ARL 67156 (100 μM), was a weaker inhibitor of ATP degradation and inhibited UTP breakdown by ~40%. Metabolism of ATP and UTP by cultured rat superior cervical ganglia was reduced after treatment with cytosine-beta-arabinoside, suggesting that non-neuronal cells along with neuronal cells contribute to their breakdown. In conclusion, these results indicate that rat superior cervical ganglia possess ecto-nucleotidases capable of catabolising purine and pyrimidine nucleotides to their nucleosides, and that dipyridamole is a potent inhibitor of ecto-nucleotidase activity.

Keywords: ATP; Ecto-nucleotidase; Dipyridamole; Ecto-ATPase; Superior cervical ganglion, rat; UTP

1. Introduction

Sympathetic ganglia have been suggested to possess distinct extracellular receptors for purine and pyrimidine nucleotides. Evidence for this hypothesis is based on studies using isolated intact rat superior cervical ganglia. On this ganglion, uridine 5′-triphosphate (UTP) is far more potent than adenosine 5′-triphosphate (ATP) or alpha, beta-methylene-ATP (a reportedly metabolically stable ATP analogue) in depolarising this tissue (Connolly et al., 1993). However, the potency of an agonist on a tissue can depend on whether it is susceptible to extracellular metabolism by the tissue (Cusack et al., 1988). The metabolism of purine and pyrimidine nucleotides by cultured rat superior cervical ganglion has yet to be reported. Here we report on studies to examine metabolism by cultured rat superior cervical ganglion of uridine and adenosine nucleotides and nucleosides. In particular, we report on the ability of dipyridamole (an inhibitor of adenosine transport), and a novel ecto-ATPase inhibitor ARL 67156 (6-*N*,*N*-diethyl-D-beta-gamma-dibromomethylene-ATP) (Crack et al., 1995) to alter the extracellular metabolism of ATP and UTP by cultured rat superior cervical ganglia.

2. Material and methods

2.1. Cell culture

Ganglia were isolated from 15–19 day old Sprague–Dawley rats, chopped and incubated with collagenase (500 U/ml) for 15 min at 37°C followed by treatment with trypsin (1 mg/ml) for 30 min at 37°C in Hanks Balanced Salt Solution without added calcium or magnesium, buffered with 10 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (HEPES) and 0.6 mg/ml bovine serum albumin. The tissue was triturated with a fire-

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polished Pasteur pipette in culture medium comprising L-15 medium, 10% Foetal Calf Serum, 24 mM $NaHCO_3$, 38 mM glucose, 2 mM glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin, nerve growth factor (7 S fraction at 25 ng/ml; TCS Biologicals) until a single cell suspension was obtained. This suspension was centrifuged at $500 \times g$ for 3 min, cells were then re-suspended in culture medium and plated at a density of 500 cells (0.5 ml) on laminin (10 μg/ml) coated multi-well tissue culture dishes. Cells in culture media, were placed in an incubator at 37°C (high humidity, 5% CO_2, 95% air), and the medium changed every 3 days. Where specified superior cervical ganglion cell cultures were treated after 1 day with 10 μM cytosine-beta-arabinoside for 2–3 days to kill off dividing cells, and then changed to normal culture medium. All cultures were used within 6 to 13 days of preparation.

2.2. Metabolism studies

Before each experiment cells were washed twice with buffered physiological salt solution containing (mM): HEPES 10, NaCl 120, $NaHCO_3$ 22.6, KCl 3, $MgCl_2$ 1.2, glucose 11, $CaCl_2$ 2.5; pH 7.4) and 500 μl of this solution added to each well. Tissue culture dishes were placed in a gently agitating chamber (well temperature 25 ± 1°C) within an enclosed environmental chamber saturated with 5% CO_2/95% O_2 and allowed to pre-equilibrate for 35 min. 50-μl samples of physiological salt solution were then taken from each well and 50 μl of purine or pyrimidine nucleotide (final concentration approximately 80 μM) added to each well (time = 0 min). After 1, 30 and 60 min, 50 μl samples were taken from each well into microcentrifuge tubes, immediately placed on ice, and subsequently frozen at −70°C until assayed by high performance liquid chromatography (HPLC). Culture wells containing ATP or UTP in physiological salt solution but without cells were incubated in order to study nucleotide stability and these results are referred to as 'blank' experiments. The effects of ARL 67156 (final concentration 100 μM) or dipyridamole (10 μM) were determined after pre-incubating cells for 35 min with either compound dissolved in buffered physiological salt solution. Where specified, N^1-phenyl-N^4-dimethyl-piperazinium iodide (DMPP) (100 μM) was applied at time zero, after pre pre-incubating cells with dipyridamole (10 μM) for 35 min.

2.3. Drugs

Drugs were dissolved in buffered physiological salt solution and frozen (−20°C) until use. Dipyridamole was prepared by adding 100 μl of 1 M hydrochloric acid to 6–10 mg of drug and diluting this into buffered physiological salt solution at a final concentration of 10 μM. Dipyridamole addition did not alter the pH of the physiological salt solution. Adenosine hemisulphate and all other compounds except where stated were obtained from Sigma. 2-Methylthio-ATP was purchased from Research Biochemicals and salts for physiological salt solution were of analytical grade. Tissue culture reagents and plastics were from Life Technology or Sigma. Nerve Growth Factor (NGF) was generously provided by TCS Biologicals (Alomone). ARL 67156 was kindly donated by Dr. Paul Leff (Astra-Charnwood).

2.4. HPLC assay of purine and pyrimidine nucleotides and nucleosides

Separation and identification of purine and pyrimidine nucleotides and nucleosides in physiological salt solution media was achieved using HPLC (Waters, 'Millennium' system). An anion exchange column (250 × 3.2 mm amino, Phenomenex, UK) was equilibrated with 5 mM potassium dihydrogen orthophosphate (pH 2.85). Over 30 min a linear gradient (0% to 40%) of 500 mM potassium dihydrogen orthophosphate with 500 mM potassium chloride (pH 3.2) was used to elute the nucleotides and nucleoside components (Simmonds et al., 1991). Peaks in samples were identified by comparison to known standards for their characteristic retention times and by dual channel detection and their ratios at 254 nm and 280 nm. The method allowed clear separation and identification of adenosine, guanosine and pyrimidine nucleotides, as well as inosine 5′-monophosphate. The nucleosides uridine and adenosine were detectable at the early phase of the chromatogram, but the presence of inosine and bases such as hypoxanthine could not be confidently assessed.

2.5. Statistical analysis

Experimental data are presented as mean ± S.E.M. and where the error was smaller than the symbol it is hidden within the symbol. All analysis was performed on raw non-transformed data. Differences between metabolite concentrations with time and in the absence or presence of dipyridamole or ARL 67156 were analysed for statistically significant changes using paired and unpaired Student's *t*-tests (two tailed) as appropriate, and $P < 0.05$ were considered significant ($n = 3$ to 5 wells/culture).

3. Results

3.1. Extracellular metabolism of ATP and ADP

There was no detectable release of adenine nucleotides, adenosine or hypoxanthine into the physiological salt solution medium (limits of detection ~1 μM) from cultures during the preincubation period (data not shown). Contamination of commercially available ATP by ADP and AMP was negligible (< 1%). In the absence of cells, i.e., 'blank'

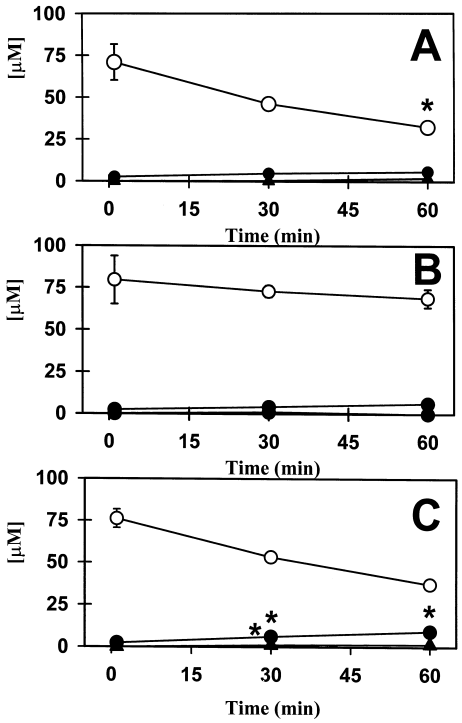

Fig. 1. Metabolism of ATP (open circle) and formation of ADP (closed circle) and AMP (closed triangle) by cultured superior cervical ganglion in (A) physiological salt solution, (B) with dipyridamole (10 μM) and (C) with ARL 67156 (100 μM). $n = 3$ to 5 wells per experiment performed in duplicate (A) or triplicate (B,C), $P < 0.05$ indicated by *.

experiments, there was no spontaneous production of ADP and AMP from ATP during incubation in physiological salt solution. Extracellular ATP was rapidly metabolised by cultured superior cervical ganglion cells (Fig. 1A). However, this did not result in a proportionate increase in ADP and AMP, i.e., there was an overall loss of total adenosine nucleotides from the physiological salt solution, as can be readily seen in Fig. 1A and C. During all studies of the metabolism of ATP, no evidence could be found for the accumulation of inosine 5′-monophosphate in physiological salt solution, suggesting the absence of an ecto-AMP deaminase.

In the presence of dipyridamole the breakdown of ATP by superior cervical ganglion was not significant (Fig. 1B), i.e., nearly 100% inhibition, similar to the cell-free 'blank' experiments. ARL 67156 reduced the breakdown of ATP by less than 15% (Fig. 1C).

3.2. Response of cultured superior cervical ganglion to a nicotinic agonist

In the absence of DMPP but presence of dipyridamole, i.e., during the pre-incubation period, there was no detectable release of adenosine nucleotides or adenosine into physiological salt solution (data not shown). In contrast, DMPP in the presence of dipyridamole caused the release of adenine nucleotides into the bathing solution, i.e., ADP and AMP and also lead to significant accumulation of adenosine (Fig. 2).

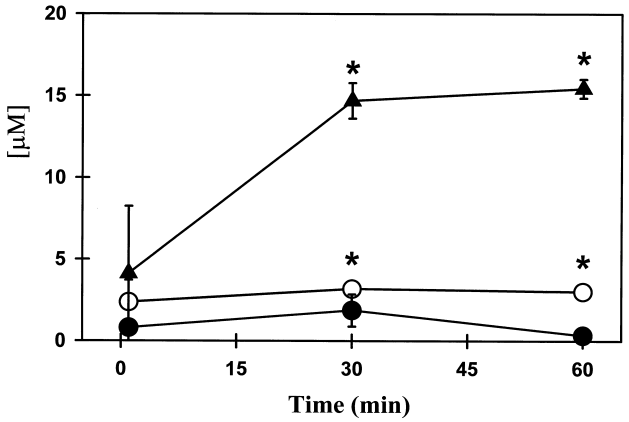

Fig. 2. Effect of the nicotinic receptor agonist, N^1-phenyl-N^4-dimethyl-piperazinium iodide (DMPP at 100 μM) on cultured superior cervical ganglion recorded in the presence of the adenosine uptake inhibitor dipyridamole (10 μM). DMPP was applied for the duration of the incubation. Adenosine (closed triangles), ADP (open circles) and AMP (closed circles). $n = 3$ to 5 wells in one experiment, $P < 0.05$ indicated by *.

3.3. Metabolism of ATP analogues, 2-methylthioATP and alpha, beta-methylene-ATP

Commercially available 2-methylthioATP was found to contain approximately 21% contamination by the diphosphate and 2% of the monophosphate. Cultured superior cervical ganglion were found to metabolise 50% of the 2-MethylthioATP during 60 min. ($P < 0.05$), with a concomitant increase in the monophosphate, whereas the diphosphate remained constant (Fig. 3A). Alpha, beta-methylene-ATP contained less than 1% alpha, beta-meth-

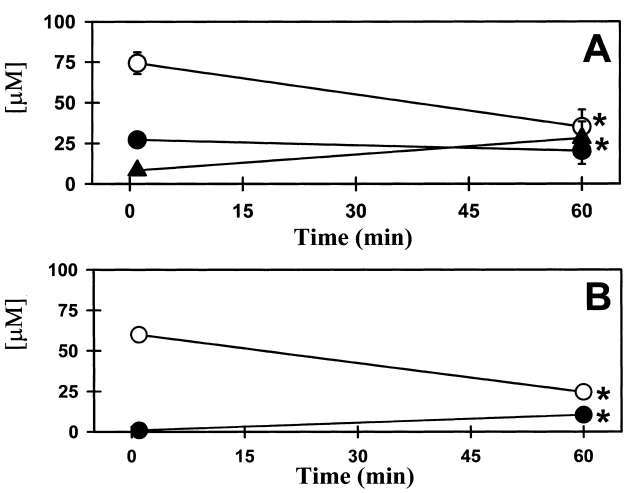

Fig. 3. Metabolism by cultured superior cervical ganglion in physiological salt solution. (A) metabolism of 2-methyl-thioATP (open circle) to 2-methyl-thioADP (closed circles) and 2-methyl-thioAMP (closed triangles) and (B) metabolism of alpha,beta-methylene-ATP (open circles) and formation of alpha,beta-methylene-ADP (closed circles). $n = 3$ or 4 wells per experiment performed in duplicate, $P < 0.05$ indicated by *.

ylene-ADP and other purine contaminants. Superior cervical ganglion rapidly catabolised alpha, beta-methylene-ATP to its diphosphate but not to the monophosphate (Fig. 3B).

3.4. Metabolism of UTP

Commercially available UTP contained about 20% UDP, with a trace of UMP (approx. 5%) and no uridine. Neither UTP nor UDP (nor UMP) concentrations in the physiological salt solution alone (i.e., 'blank') changed during incubation for up to 60 min (data not shown).

Superior cervical ganglion cells metabolised UTP to UDP, UMP and uridine (Fig. 4A), these changes being approximately additive, i.e., there was no change in total pyrimidine content of the physiological salt solution. In the presence of dipyridamole, breakdown of UTP and UDP was effectively inhibited, by almost 100% (Fig. 4B), while ARL 67156 reduced both UTP and UDP breakdown by approximately 40% (c.f. Fig. 4A and C).

3.5. Cytosine-beta-arabinoside effects

Non-neuronal cells, i.e., 'support cells' such as glia, were prevented from proliferating by treating superior cervical ganglion cultures with cytosine-beta-arabinoside. Using cytosine-beta-arabinoside treated cultures, the breakdown of ATP and UTP (Fig. 5) in both cases was reduced by approximately 50% (c.f. Figs. 1A, 2A and Fig. 3). These results suggest that non-neuronal cells contribute overall to the breakdown of these nucleotides along with neuronal cells.

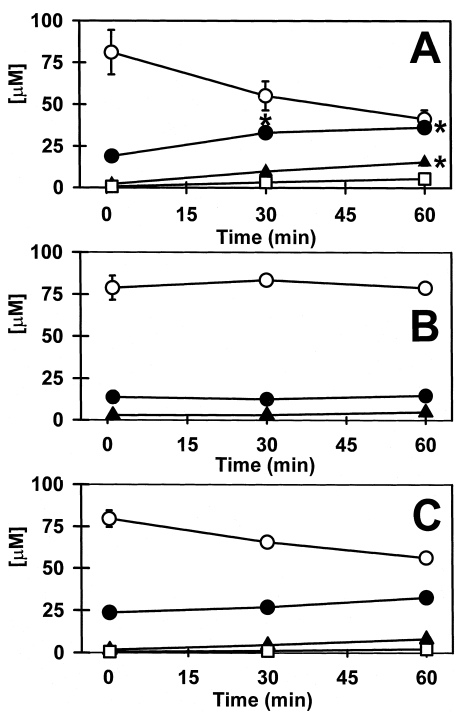

Fig. 4. Metabolism of UTP (open circles) and formation of UDP (closed circles), UMP (closed triangles) and uridine (open squares) by cultured superior cervical ganglion in (A) physiological salt solution, (B) with dipyridamole (10 μM), and (C) with ARL 67156 (100 μM). $n = 4$ wells per experiment performed in duplicate, $P < 0.05$ indicated by *.

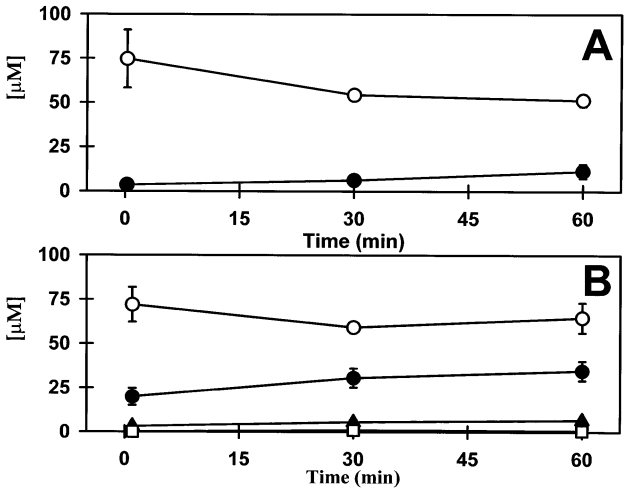

Fig. 5. (A) Metabolism of ATP (open circles) and formation of ADP (closed circles) by cultured superior cervical ganglion pre-treated with cytosine-beta-arabinoside; (B) Metabolism of UTP (open circles) and formation of UDP (closed circles) and UMP(closed triangles) and uridine (open squares) by cultured superior cervical ganglion pre-treated with cytosine-beta-arabinoside. $n = 4$ wells per experiment performed in duplicate.

4. Discussion

4.1. ATP breakdown

Cultured rat superior cervical ganglia readily catabolised ATP and UTP to their di- and monophosphates by ecto-nucleotidase activity. UMP was further metabolised to uridine, which could be measured by HPLC. AMP did not appear to be metabolised to inosine 5′-monophosphate extracellularly by adenylate deaminase. However, there was circumstantial evidence, i.e., the loss of total adenosine nucleotides, that AMP was further broken down by ecto-5′-nucleotidase to adenosine, although this metabolite did not accumulate in the physiological salt solution. Adenosine could have been either taken up by the cells, or further metabolised to inosine by an ecto-adenosine deaminase.

The accumulation of adenosine in the DMPP-stimulated cultures suggested that dipyridamole prevented the uptake of adenosine into cultured superior cervical ganglion cells, and was consistent with its ability to enhance the adenosine-induced hyperpolarisation of intact rat superior cervical ganglia (Connolly and Stone, 1993) and other tissues. Support for the presence of purine ecto-5′-nucleotidase activity on intact rat superior cervical ganglia using cytochemical studies was reported by Nacimento and

Kreutzberg (1990), who found its presence on capillaries and absence on the neuropile. Subsequently, Vizi et al. (1997) found good evidence for the presence of ecto-ATPase activity in intact rat superior cervical ganglia, both enzymatically and by histochemical methods. The presence of an uptake system for adenosine, and possibly an ecto-adenosine deaminase activity, in intact rat superior cervical ganglia (Connolly and Stone, 1993) is also consistent with the expression of enzymes capable of extracellular catabolism of ATP.

Importantly, we found that ATP breakdown was almost totally inhibited by micromolar concentrations dipyridamole, which is both an effective adenosine uptake inhibitor at micromolar concentration and an inhibitor of phosphodiesterase activity at around 40-fold higher concentrations (Weishaar et al., 1985). In contrast, ARL 67156, a putative ATPase inhibitor (Crack et al., 1995), was a poor inhibitor of ATP degradation by superior cervical ganglia ecto-nucleotidase activity. The possibility that superior cervical ganglia contain ecto-enzymes other than ATPase, such as an ecto-apyrase, is worthy of further investigation using procedures such as described by Smith et al. (1999).

Alpha, beta-methylene-ATP was rapidly metabolised to alpha, beta-methylene-ADP, a known inhibitor of ecto-5′-nucleotidase and hence inhibitor of its own breakdown (Cusack et al., 1988). This also demonstrated that the ecto-nucleotidase activity we observed acts sequentially from the terminal phosphate position. The lack of production of adenosine from alpha, beta-methylene-ATP/ADP suggests the absence of an ecto-pyrophosphatase, which might by-pass the methylene bond. The breakdown of alpha,beta-methylene-ATP to alpha,beta-methylene-ADP was more rapid than that reported for other tissues, which nonetheless are often studied at the higher temperature of 37°C to increase catabolic rates. Thus, using guinea pig taenia coli muscle tissue, Welford et al. (1986) found alpha,beta-methylene-ATP was extremely slowly degraded. We suggests caution in the use of alpha,beta-methylene-ATP as a 'metabolically stable' ATP analogue until its metabolism is known for the tissue under study. We reported that on intact rat superior cervical ganglion, alpha, beta-methylene-ATP was less potent than UTP (Connolly et al., 1993). However, because both compounds have been shown to be subject to metabolism by ecto-nucleotidase in this study, further assessment of their potency (i.e., in the presence of ecto-nucleotidase inhibitors) or the use of more metabolically stable analogues of ATP and UTP, is now required.

4.2. Effect of DMPP on cultured superior cervical ganglia

The ability of DMPP to cause the release of adenosine nucleotides and adenosine but not uridine nucleotides is an intriguing observation. The release of purines upon depolarisation of cultured superior cervical ganglion neurones by 'high potassium' has been reported by Wolinsky and Patterson (1985). It has been reported that carbachol, a muscarinic and nicotinic receptor agonist, caused release of ATP from intact superior cervical ganglia (Vizi et al., 1997). This is a finding consistent with the present study showing the release of adenine nucleotides and adenosine in media containing dipyridamole. DMPP activates nicotinic receptors on post-ganglionic superior cervical ganglion neurones to cause their depolarisation, which could result in the release of ATP (or ADP). These nucleotides may then activate post-synaptic P2 purinoceptors to potentiate the nicotinic actions of DMPP (i.e., acetylcholine release in vivo) followed by extracellular metabolism to adenosine which may act to inhibit further postsynaptic activation, i.e., by depression of muscarinic receptors activated by acetylcholine (see Connolly et al., 1993; Connolly and Stone, 1995). In addition, a retrograde action of adenine nucleosides and adenosine on pre-synaptic nerve terminals could also occur.

4.3. UTP breakdown

UTP was degraded by superior cervical ganglia cell ecto-nucleotidase to form UDP, UMP and uridine, these products accumulated in the incubation media in a manner that suggested there was no further metabolism or uptake of uridine by superior cervical ganglia comparable to that occurring with adenosine. Dipyridamole was a powerful inhibitor of UTP breakdown by superior cervical ganglion ecto-nucleotidases, similar to its action with ATP. ARL 67156 was a more potent inhibitor of the breakdown of UTP than ATP.

4.4. Ecto-nucleotidase inhibitors

From the results presented here we suggest that at least one extracellular enzyme on superior cervical ganglion cells, i.e., a non-specific ecto-nucleotidase, catabolises both ATP and UTP. This is also consistent with our observations reported for intact superior cervical ganglion, which catabolised both ATP and UTP (see Connolly et al., 1998). The unexpectedly powerful inhibition of both ATP and UTP breakdown by dipyridamole supports this observation. The similar potency of degradative enzymes on rat peripheral nervous tissues for ATP and UTP is consistent with a lack of selectivity of ecto-nucleotidase for ATP or UTP present on other tissues, including those of the nervous system (Stefanovic et al., 1976). The ability of ecto-nucleotidases to metabolise both ATP and UTP also suggests that the notably higher potency of UTP compared to ATP in depolarising isolated superior cervical ganglia (Connolly, 1994) is not due to selective catabolism of ATP versus UTP.

The mechanism for dipyridamole inhibition remains unknown and further study is suggested. Interestingly, dipyridamole has been reported not to affect the final step

in the breakdown of AMP by cardiac sarcolemmal 5′-nucleotidase (Heyliger et al., 1981), suggesting that it may have a selective action on neuronal ecto-nucleotidase. Lai and Wong (1991), using central nervous system astrocytes, reported that catabolism of ATP to ADP, AMP and adenosine was not inhibited by 20 μM dipyridamole. Our results using 10 μM dipyridamole therefore indicate a differential effect between central nervous system and peripheral nervous system glial cells such as rat astrocytes, where an ecto-nucleotidase exists (Lai and Wong, 1991), and the ecto-ezymes on human astrocytoma cells (Lazarowski et al., 1997). Thus, we suggest that dipyridamole probably inhibits the first step, i.e., ATP/UTP breakdown by ecto-nucleotidase but may not affect AMP 5′-nucleotidase. A further possibility is that the end-product of ATP catabolism, adenosine, may act as a feed-back inhibitor of nucleotide degradation, however, such a mechanism is known not to exist intracellularly, and would be unexpected for ecto-enzymes acting in the extracellular space. In addition, the adenosine uptake inhibitor *S*-(4-nitrobenzyl)-6-thioinosine at 10 μM had no detectable effect on extracellular metabolism of ATP by the rat colon muscularis (Tennant et al., 1999).

The action of the experimental 'ATPase inhibitor' ARL 67156 suggests that this drug is an effective inhibitor of UTP breakdown by superior cervical ganglion cells in contrast with its weaker suppression of ATP catabolism. Using rabbit isolated tracheal epithelium, responses to UTP but not ATP-γ-S (a metabolically stable analogue) were potentiated by ARL 67156 (Crack et al., 1995), suggesting ARL 67156 inhibits breakdown of UTP. ARL 67156 is also an effective inhibitor of the catabolism of ATP by human erythrocytes (Crack et al., 1995), and to a lesser extent by isolated rat superior cervical ganglion and vagi (Connolly et al., 1998). Interestingly, our previous study with intact superior cervical ganglion found that ARL 67156 inhibited breakdown of ATP more effectively than UTP (Connolly et al., 1998). The reverse finding here is consistent with a contributing action by support cells of intact superior cervical ganglia (Fig. 6).

From the published literature and our own observations, we suggest that different ecto-nucleotidases can exist on the same or on distinct cell types. In Fig. 6, we propose a possible model which is consistent with the observations reported here and in our previous paper using intact rat superior cervical ganglia, nodose ganglia and vagi (Connolly et al., 1998). We suggest that the metabolism of ATP and UTP by neurones and by support cells, e.g., glia, is regulated by the expression of a single ecto-nucleotidase on neurones (termed 'En'), which catabolises ATP and UTP at a similar rate (c.f. Fig. 1A and 4A), is strongly inhibited by dipyridamole (Fig. 1B and 4B), but is only weakly antagonised by ARL 67156 (Fig. 1B and 4B). In contrast, glia possess both the neuronal ecto-nucleotidase enzyme activity, i.e., En, as well as a second ecto-nucleotidase enzyme (termed 'E' in Fig. 6), which catabolises UTP and is inhibited equally by dipyridamole and ARL 67156. Thus, intact superior cervical ganglion which contain more non-neuronal cells (glia, endothelial cells) versus neurones, metabolise ATP and UTP via 'En' and 'E', and thus is partially inhibited by ARL 67156.

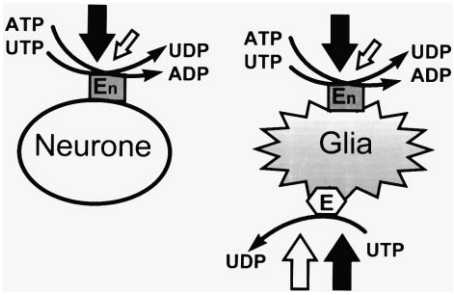

Fig. 6. Model depicting the metabolism of ATP and UTP by neurones and by support cells, e.g., glia. N.B. Neurones possess a single ecto-nucleotidase termed En, that catabolises ATP and UTP at a similar rate and is strongly inhibited by dipyridamole (solid arrow) but only weakly by ARL 67156 (open arrow). In contrast, glia possesses two enzymes, namely En, found on neurones and a second enzyme termed E, which catabolises UTP and is inhibited equally by dipyridamole and ARL 67156. The size of the arrow indicates the degree of inhibitory activity.

Superior cervical ganglion cells in culture and treated with cytosine-beta-arabinoside, are nearly pure neuronal cultures, and thus catabolism of ATP and UTP in these cells would be predicted to be poorly inhibited by high concentrations of ARL 67156 but strongly inhibited by dipyridamole (Fig. 1B,C and 4B,C). This model also explains why catabolism of ATP by intact nodose ganglia is not inhibited by ARL 67156 (Connolly et al., 1998), because it contains predominately neuronal cell bodies which are predicted to express the ecto-enzyme 'En'. Erythrocyte ecto-nucleotidase, which is insensitive to dipyridamole (Crack et al., 1995), would be predicted to be different to 'E' and 'En'.

Dipyridamole is used by pharmacologists mainly to inhibit uptake of adenosine in experiments designed to understand how purines and pyrimidines activate extracellular receptors for these compounds. Dipyridamole was at least 10-fold more potent than ARL 67156 in blocking the breakdown of ATP and UTP by superior cervical ganglia cells. This inhibitory effect of dipyridamole was quite remarkable, as in this study we used high but physiological concentrations of ATP and UTP. Dipyridamole may antagonise nucleotide catabolism at sub- or micromolar concentrations, which are below concentrations required to antagonise adenosine uptake. In addition, dipyridamole is used clinically as a potent coronary vasodilator, as a coactivator for some antitumor drugs, for stroke prevention and as an anti-platelet aggregation drug. Hence, some of these effects may be mediated via an inhibition of ecto-nucleotidease, with or without inhibition of adenosine transport. Thus, dipyridamole or one of its structural analogues might prove to be an interesting lead compound for development as a 'novel' ecto-nucleotidase inhibitor.

5. Conclusion

Our observations are fully consistent with our previous observations that sympathetic ganglia such as the rat superior cervical ganglion possess ectoanucleotidases capable of catabolising purine and pyrimidine nucleotides to their nucleosides, and a mechanism for the uptake of adenosine but not uridine (Connolly and Stone, 1993; Connolly et al., 1998). These observations are also consistent with the release of extracellular purines and pyrimidines and their hypothesised role as regulators of the peripheral nervous system (Connolly et al., 1993, 1998).

The obvious contamination of some commercial nucleotide preparations but not others confirms our previous suggestion that the purity of purine and pyrimidines used for pharmacological research need to be assessed, or taken into account in experimental situations (Connolly et al., 1997, 1998).

ARL 67156 in this study proved to be a inhibitor of both ATP and UTP catabolism, showing slight selectivity for UTP over ATP, and thus may not be solely an ecto-ATPase inhibitor. The novel finding that dipyridamole is a potent inhibitor of ecto-nucleotidase warrants further detailed study.

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