Adenosine Receptor Activation and the Regulation of Tyrosine Hydroxylase Activity in PC12 and PC18 Cells

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Abstract: We compared the response of rat PC12 cells and a derivative PC18 cell line to the effects of adenosine receptor agonists, antagonists, and adenine nucleotide metabolizing enzymes. We found that theophylline (an adenosine receptor antagonist), adenosine deaminase, and AMP deaminase all decreased basal cyclic AMP content and tyrosine hydroxylase activity in the PC12 cells, but not in PC18 cells. Both cell lines responded to the addition of 2-chloroadenosine and 5'-N-ethylcarboxamidoadenosine, adenosine receptor agonists, by exhibiting an increase in tyrosine hydroxylase activity and cyclic AMP content. The latter finding indicates that both cell lines contained an adenosine receptor linked to adenylate cyclase. We found that the addition of dipyridamole, an inhibitor of adenosine uptake, produced an elevation of cyclic AMP and tyrosine hydroxylase activity in both cell lines. Deoxycoformycin, an inhibitor of adenosine deaminase, failed to alter the levels of cyclic AMP or tyrosine hydroxylase activity. This suggests that uptake was the primary inactivating mechanism of adenosine action in these cells. We conclude that both cell types generated adenine nucleotides which activate the adenosine receptor in an autocrine or paracrine fashion. We found that PC12 cells released ATP in a calcium-dependent process in response to activation of the nicotinic receptor. We also measured the rates of degradation of exogenous ATP, ADP, and AMP by PC12 cells. We found that the rates of metabolism of the former two were at least an order of magnitude greater than that of AMP. Any released ATP would be rapidly metabolized to AMP and then more slowly degraded to adenosine. That this situation reflects metabolism in vivo in the PC12 cells is suggested by the finding that AMP deaminase was more effective than adenosine deaminase in decreasing basal cyclic AMP and tyrosine hydroxylase activity. Key Words: Adenosine receptors—Tyrosine hydroxylase—Cyclic AMP—PC12 cells—PC18 cells—Rat. Roskoski R. Jr. and Roskoski L. M. Adenosine receptor activation and the regulation of tyrosine hydroxylase activity in PC12 and PC18 cells. J. Neurochem. 53, 1934-1940 (1989).

Erny and co-workers (1981) reported that cells obtained from a transplantable rat pheochromocytoma respond to 2-chloroadenosine with an increase in cyclic AMP production and an increase in tyrosine hydroxylase activity. These studies were extended to PC12 cells in culture (Rabe and McGee, 1983; Erny and Wagner, 1984). Erny and Wagner (1984) showed that adenosine receptor agonists and cholera toxin (an activator of adenylate cyclase) increase cyclic AMP levels and activate tyrosine hydroxylase. Erny et al. (1981) showed that adenosine deaminase decreases basal tyrosine hydroxylase activity in cell suspensions prepared from the solid tumor. Tyrosine hydroxylase is phosphorylated and activated by several protein kinases, including cyclic AMP-dependent protein kinase (Campbell et al., 1986).

Burnstock (1978) classified purine receptors into two classes based upon agonist potency and the effects of methylxanthine antagonists, such as theophylline. The P1 receptor is inhibited by theophylline, and the order of agonist potency is adenosine > AMP > ADP > ATP. The P2 receptor is less sensitive to the action of theophylline, and the order of agonist potency is reversed (ATP > ADP > AMP > adenosine). The P1 receptor is composed of two subclasses now called A1 and A2 (Williams, 1987). Adenosine A1 receptors are usually, but not exclusively, linked to inhibition of adenylate cyclase. Adenosine A2 receptors are usually, but not exclusively, linked to activation of adenylate cyclase. Although they can be distinguished by the potency of pharmacological agonists and antagonists, both are activated by 5'-N-ethylcarboxamidoadenosine (NECA).

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Abbreviations used: DMPP, 1,1-dimethyl-4-phenylpiperazinium iodide; NECA, 5'-N-ethylcarboxamidoadenosine.
and 2-chloroadenosine (Williams, 1987). Williams et al. (1987) demonstrated that PC12 cells contain an adenosine receptor with A2 selectivity. The ability of adenosine deaminase (Enny et al., 1981) and theophylline (Roskoski and Roskoski, 1987) to decrease basal tyrosine hydroxylase activity in PC12 cells suggests that the A2 receptor is activated by endogenously released compounds under nonstimulatory conditions. Sattin and Rall (1970) were the first to describe the actions of adenosine on the activation of guinea pig brain adenylylate cyclase. The methylxanthines, including caffeine and theophylline, are A1 and A2 receptor antagonists, and this fact lead to the proposal that the central stimulant action of caffeine might be due to the blockade of the sedative actions of endogenous adenosine (Sattin and Rall, 1970).

We addressed the source of the natural agonist in PC12 cells which produces the steady-state activation of tyrosine hydroxylase that is inhibited by theophylline. One possibility is that ATP is released by exocytosis and is metabolized to adenosine (Burnstock and Kennedy, 1986). ATP occurs in both cholinergic and adrenergic vesicles (Winkler, 1977; Zimmerman, 1978), and both are present in PC12 cells (Greene and Tischler, 1982; Wagner, 1985). A second, but not mutually exclusive, possibility is that adenosine per se is released. Based on comparative studies with adenosine deaminase and AMP deaminase, we found that AMP affected tyrosine hydroxylase activity in PC12 cells to a greater degree than does adenosine under basal conditions. We also found that dipyridamole increased the cyclic AMP levels and tyrosine hydroxylase activity in PC12 and PC18 cells. This suggests that transport of adenosine into cells was the mechanism for terminating its action.

MATERIALS AND METHODS

Stock cultures of PC12 cells and PC18 cells were maintained as described by Baizer and Weiner (1985). Cells were harvested by trituration, suspended in HEPES-Ringer solution [about 2 mg of protein/ml as subsequently determined by the Bradford (1976) procedure using bovine γ-globulin as standard], distributed in 58-μl portions following gentle vortexing, and treated with 2 μl of the designated agent or vehicle to give the final specified concentration. Incubations were for 5 min (or other specified period) at 30°C. For the cyclic AMP measurements, the tubes were placed in a boiling water bath for 5 min at the conclusion of the incubation. The samples were stored at −70°C for periods of up to 1 month prior to cyclic AMP determinations by the procedure of Harper and Brooker (1975). The samples were sonicated for 10 s and total protein was measured (Bradford, 1976). The samples were centrifuged at 13,600 g for 5 min, and the cyclic AMP in the supernatant was measured by radioimmunoassay (Harper and Brooker, 1975).

To measure the cyclic AMP-dependent protein kinase activity ratio, after incubation, the cells were centrifuged at 13,600 g for 30 s and the medium was aspirated. Then 150 μl of ice-cold phosphate buffer (Johnson and Lincoln, 1985) were added, the cells were sonicated, and the protein kinase was measured radiochemically with Kemptide (LRRASLG) as acceptor substrate with and without 10 μM cyclic AMP for 5 min as previously described (Roskoski, 1983). For tyrosine hydroxylase activity determinations in vitro, cells were treated and harvested as previously documented (Roskoski and Roskoski, 1987). Assays were performed at 30°C with saturating 6-methyluradophydrin (125 μM) at pH 6.0. Data were analyzed by Student’s t test or analysis of variance and Tukey’s test as described by Zar (1984).

To measure ATP release, PC12 cells in Ringer’s solution (about 2 mg of protein/ml) were dispensed in 58-μl portions and allowed to equilibrate for 10 min at ambient temperature in normal or calcium-free Ringer’s solution. Portions (1 μl) of hexamethonium or LaCl3 were added 10 min prior to the addition of 1 μl of 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) to give a final volume of 60 μl. Ninety seconds after the addition of DMPP, the cells were centrifuged for 30 s at 13,600 g. Then 50-μl portions of the supernatant were added to 15 μl of ice-cold 0.4 M HClO4. Portions of 50 μl were then taken for the firefly luciferase assay, with the following modifications of the assay described by Stanley and Williams (1969). Stock MgSO4 was added to the arsenate buffer prior to the measurements to eliminate precipitation of salts during long-term storage, and the assay solution contained exogenous D-luciferin (17 μM).

DMPP was purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI, U.S.A.) and LaCl3 from Fisher Scientific Co. (Fair Lawn, NJ, U.S.A.). Bovine γ-globulin was purchased from Bio-Rad Laboratories (Richmond, CA, U.S.A.). AMP deaminase, adenosine deaminase, dipyridamole, deoxycoformycin, NECA, and other chemicals were obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). [3H]ATP, [3H]ADP, [3H]AMP (all uniformly labeled), and the cyclic AMP radioimmunoassay kit were obtained from New England Nuclear Corp. (Boston, MA, U.S.A.). The PC12 cells were kindly provided by Dr. James Byrd of the National Institute of Mental Health (Washington, DC, U.S.A.), and the PC18 cells were provided by Dr. A. William Tank (University of Rochester, Rochester, NY, U.S.A.). We used PC12 cells in passages between 50 and 75; the PC18 cells were used between passages 10 and 30. The later passage PC12 and PC18 cells responded to the adenosine receptor agonists and exhibited usual tyrosine hydroxylase activity. The sources of the other materials are as previously specified (Vrana et al., 1981).

RESULTS

Effects of adenine nucleotides on cyclic AMP levels and tyrosine hydroxylase activity

The effectiveness of several agents in altering cyclic AMP levels and tyrosine hydroxylase activity in vitro following treatment of intact cells was examined. Adenosine increased cyclic AMP levels sixfold and increased hydroxylase activity about 31% (Table 1). ATP, ADP, and AMP produced a ninefold increase in cyclic AMP and about a 55% increase in tyrosine hydroxylase activity. These three nucleotides were significantly more effective than the nucleoside adenosine. NECA and 2-chloroadenosine, furthermore, were more effective than the naturally occurring adenine nucleotides, as shown in Table 1. Inosine (the deaminated metabolite of adenosine), guanosine, IMP, and exogenous

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cyclic AMP were without effect in augmenting tyrosine hydroxylase activity. Theophylline, an adenosine P1 receptor antagonist, decreased basal cyclic AMP (50%) and tyrosine hydroxylase activity (25%). Although theophylline in PC12 cells was blockade of the adenosine receptor.

We determined the time course of the changes in tyrosine hydroxylase activity in response to NECA and adenosine and found that the full activation occurred within 15 s of treatment (not shown). The effects of these agents were independent of external calcium in the medium even in the presence of either 0.1 mM EGTA, 1 mM LaCl3, or 4 mM MnCl2 (not shown).

The predominant action of cyclic AMP in eukaryotic systems is to activate its corresponding cyclic AMP-dependent protein kinase (Kuo and Greengard, 1969; Nestler and Greengard, 1984). Following treatment of the PC12 cells with the specified agents for 5 min. we measured cyclic AMP-dependent protein kinase activity under the conditions described by Johnson and Lincoln (1985). The assays were performed with and without 10 μM cyclic AMP, and the results are expressed as the protein kinase activity ratio (Corbin, 1983). We found that the adenosine nucleotides activated this enzyme as expected, because of their ability to elevate cyclic AMP levels (Table 1). The increases in the protein kinase activity ratio paralleled the increases in tyrosine hydroxylase activity. We also found that theophylline decreased this activity ratio.

A comparison of the effects of adenosine deaminase and AMP deaminase treatment of PC12 and PC18 cells

Erny and Wagner (1984) treated PC12 cells with adenosine deaminase to decrease the basal levels of cyclic AMP. The action of this enzyme and also theophylline is to abrogate the effects of endogenously generated adenosine and adenosine nucleotides on the adenosine receptor which, in this system, is coupled to adenylate cyclase in a stimulatory fashion. The PC18 cell line, which was derived from the PC12 cells, fails to respond to nerve growth factor, in contrast to the parent cell line (Tank et al., 1986). We wanted to compare responses of the two cell lines to agents expected to alter adenosine action and metabolism. Unlike the PC12 cells, the PC18 cells failed to respond to the addition of theophylline or adenosine deaminase by decreasing cyclic AMP levels or by decreasing tyrosine hydroxylase activity (Table 2). We also found that AMP deaminase in PC12 cells was more effective in decreasing the basal cyclic AMP levels and tyrosine hydroxylase activity than was adenosine deaminase (Table 2). We examined the effectiveness of each of these agents throughout a wide range of concentrations. Five times the specified concentration of each agent was no more effective than that shown in Table 2. AMP deaminase and adenosine deaminase had no effect on cyclic AMP levels or tyrosine hydroxylase activity in PC18 cells. We found, however, that the PC18 cells responded robustly to NECA and to 2-chloroadenosine, as did the parent cell line, by exhibiting an increase in cyclic AMP content and tyrosine hydroxylase activity.

Inactivation of endogenously generated adenosine by uptake

We next wanted to ascertain the mechanism of inactivation of the endogenously generated adenosine nucleotides. To address this issue, we used dipyrivamide, an inhibitor of adenosine transport (Nimit et al., 1981), and deoxycoformycin, an inhibitor of adenosine deaminase (Skolnick et al., 1978). We found that dipyrivamide treatment resulted in a large increase in cyclic AMP and elevation of hydroxylase activity in both PC12 and PC18 cells (Table 3). In contrast, deoxyco-

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**TABLE 1. Effects of adenine nucleotides on cyclic AMP levels and tyrosine hydroxylase activity in PC12 cells**

<table>
<thead>
<tr>
<th>Addition</th>
<th>Group</th>
<th>Cyclic AMP (pmol/mg of protein)</th>
<th>Protein kinase activity ratio</th>
<th>Tyrosine hydroxylase activity (pmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>I</td>
<td>121 ± 13a</td>
<td>0.38 ± 0.02a</td>
<td>212 ± 17b</td>
</tr>
<tr>
<td>ATP, 0.1 mM</td>
<td>II</td>
<td>1,210 ± 110a</td>
<td>0.57 ± 0.03a</td>
<td>334 ± 16b</td>
</tr>
<tr>
<td>ADP, 0.1 mM</td>
<td>II</td>
<td>1,180 ± 121a</td>
<td>0.59 ± 0.03a</td>
<td>329 ± 18b</td>
</tr>
<tr>
<td>AMP, 0.1 mM</td>
<td>II</td>
<td>1,360 ± 114a</td>
<td>0.54 ± 0.03a</td>
<td>341 ± 14b</td>
</tr>
<tr>
<td>Adenosine, 0.1 mM</td>
<td>III</td>
<td>724 ± 102a</td>
<td>0.46 ± 0.03a</td>
<td>277 ± 18b</td>
</tr>
<tr>
<td>2-Chloroadenosine, 1.0 μM</td>
<td>IV</td>
<td>2,410 ± 174a</td>
<td>0.86 ± 0.05b</td>
<td>381 ± 19b</td>
</tr>
<tr>
<td>NECA, 10 μM</td>
<td>IV</td>
<td>2,630 ± 149a</td>
<td>0.88 ± 0.06b</td>
<td>394 ± 24b</td>
</tr>
<tr>
<td>Theophylline, 100 μM</td>
<td>V</td>
<td>69 ± 8a</td>
<td>0.29 ± 0.02a</td>
<td>164 ± 11b</td>
</tr>
<tr>
<td>Inosine, 100 μM</td>
<td>I</td>
<td>121 ± 14a</td>
<td>0.38 ± 0.03a</td>
<td>221 ± 19b</td>
</tr>
<tr>
<td>Guanosine, 100 μM</td>
<td>I</td>
<td>133 ± 18a</td>
<td>0.39 ± 0.03a</td>
<td>217 ± 12b</td>
</tr>
<tr>
<td>IMP, 100 μM</td>
<td>I</td>
<td>117 ± 12a</td>
<td>0.40 ± 0.02a</td>
<td>222 ± 17b</td>
</tr>
<tr>
<td>Cyclic AMP, 100 μM</td>
<td>I</td>
<td>----</td>
<td>0.39 ± 0.04a</td>
<td>215 ± 16b</td>
</tr>
</tbody>
</table>

PC12 cells were harvested, treated for 5 min with the specified agents, and taken for the various determinations as described in Materials and Methods. The values represent the means ± SEM of quadruplicate determinations of a single experiment. Similar results were obtained in four to six other experiments, depending upon the agent.

*a* Significantly different from all other groups (p < 0.01) by analysis of variance.

*b* Significantly different from all other groups (p < 0.05) by analysis of variance.
the PC18 cells also show that they produced endogenous adenosine deaminase abolished the stimulatory effects of dipyridamole (Table 3). The results indicate that the uptake of adenosine was the chief, and perhaps exclusive, mechanism for inactivating the adenosine deaminase were examined. We therefore performed experiments to determine whether PC12 cells are able to release ATP by exocytosis. The time course of the effects of a nicotine receptor agonist, DMPP, on the extracellular ATP concentration was determined. DMPP produced an increase in extracellular ATP which was seen at 1 min (the earliest time possible with a centrifugation methodology). The concentration was elevated three- to fourfold at 2 min and returned to control levels by 4 min (Fig. 1). The effect of 10 μM DMPP was abolished by 2 mM hexamethonium (Table 4). The addition of LaCl₃, a calcium antagonist, also inhibited the DMPP-stimulated ATP release (Table 4). The basal and evoked release were associated with submicromolar ATP concentrations in the extracellular fluid.

Characterization of ATP release from PC12 cells

ATP is a component of both adrenergic and cholinergic vesicles (Winkler, 1977; Zimmerman, 1978) and is associated with dopamine-containing secretory vesicles purified from PC12 cells (Wagner, 1985). One possible source of extracellular adenine nucleotide might be from the co-release of ATP from the latter vesicles. We therefore performed experiments to determine whether PC12 cells are able to release ATP by exocytosis. The time course of the effects of a nicotine receptor agonist, DMPP, on the extracellular ATP concentration was determined. DMPP produced an increase in extracellular ATP which was seen at 1 min (the earliest time possible with a centrifugation methodology). The concentration was elevated three- to fourfold at 2 min and returned to control levels by 4 min (Fig. 1). The effect of 10 μM DMPP was abolished by 2 mM hexamethonium (Table 4). The addition of LaCl₃, a calcium antagonist, also inhibited the DMPP-stimulated ATP release (Table 4). The basal and evoked release were associated with submicromolar ATP concentrations in the extracellular fluid.

Table 2. Comparison of responses of PC12 and PC18 cells to adenosine receptor agonists, antagonists, and metabolizing enzymes

<table>
<thead>
<tr>
<th>Addition</th>
<th>Cyclic AMP (pmol/mg) PC12 (group)</th>
<th>Cyclic AMP (pmol/mg) PC18</th>
<th>Tyrosine hydroxylase activity (pmol/min/mg) PC12 (group)</th>
<th>Tyrosine hydroxylase activity (pmol/min/mg) PC18</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>154 ± 12 (I)</td>
<td>44 ± 3</td>
<td>264 ± 14 (I)</td>
<td>117 ± 9</td>
</tr>
<tr>
<td>Theophylline, 100 μM</td>
<td>64 ± 8 (II)</td>
<td>49 ± 4</td>
<td>149 ± 9 (II)</td>
<td>113 ± 6</td>
</tr>
<tr>
<td>AMP deaminase, 4 U/ml</td>
<td>73 ± 7 (II)</td>
<td>42 ± 5</td>
<td>154 ± 10 (II)</td>
<td>108 ± 9</td>
</tr>
<tr>
<td>Adenosine deaminase, 4 U/ml</td>
<td>103 ± 9 (III)</td>
<td>49 ± 6</td>
<td>192 ± 9 (III)</td>
<td>114 ± 7</td>
</tr>
<tr>
<td>2-Chloroadenosine, 1.0 μM</td>
<td>1.91 ± 141 (IV)</td>
<td>714 ± 81</td>
<td>351 ± 31 (IV)</td>
<td>229 ± 14</td>
</tr>
<tr>
<td>NECA, 10 μM</td>
<td>1.720 ± 163 (IV)</td>
<td>804 ± 70</td>
<td>364 ± 27 (IV)</td>
<td>241 ± 19</td>
</tr>
</tbody>
</table>

PC12 or PC18 cells were harvested, treated for 5 min with the specified agents, and taken for cyclic AMP or tyrosine hydroxylase activity determinations as described in Materials and Methods. The data represent the means ± SEM of quadruplicate determinations of a single experiment. Similar results were obtained in four independent studies.

*Significantly different from other PC12 cell groups by analysis of variance (p < 0.01).

*Significantly different from other PC12 cell groups by analysis of variance (p < 0.05).

*Significantly different from the PC18 cell control (no addition) by Student's t test (p < 0.01).

Table 3. Effects of dipyridamole and deoxycorticosterone on cyclic AMP levels and tyrosine hydroxylase activity in PC12 and PC18 cells

<table>
<thead>
<tr>
<th>Addition</th>
<th>Cyclic AMP (pmol/mg) PC12</th>
<th>Cyclic AMP (pmol/mg) PC18</th>
<th>Tyrosine hydroxylase activity (pmol/min/mg) PC12</th>
<th>Tyrosine hydroxylase activity (pmol/min/mg) PC18</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>119 ± 8</td>
<td>63 ± 8</td>
<td>227 ± 17</td>
<td>112 ± 7</td>
</tr>
<tr>
<td>Dipyridamole, 20 μM</td>
<td>1.910 ± 121</td>
<td>1.480 ± 93</td>
<td>347 ± 24</td>
<td>221 ± 15</td>
</tr>
<tr>
<td>Deoxycorticosterone, 50 μM</td>
<td>111 ± 9</td>
<td>61 ± 7</td>
<td>219 ± 14</td>
<td>119 ± 8</td>
</tr>
<tr>
<td>Dipyridamole, 20 μM, plus deoxycorticosterone, 50 μM</td>
<td>2.010 ± 147</td>
<td>1.430 ± 85</td>
<td>332 ± 21</td>
<td>238 ± 16</td>
</tr>
<tr>
<td>Adenosine deaminase, 4 U/ml</td>
<td>71 ± 8</td>
<td>67 ± 7</td>
<td>153 ± 11</td>
<td>119 ± 6</td>
</tr>
<tr>
<td>Dipyridamole, 20 μM, plus adenosine deaminase, 4 U/ml</td>
<td>83 ± 7</td>
<td>71 ± 9</td>
<td>162 ± 9</td>
<td>124 ± 14</td>
</tr>
<tr>
<td>Ethanol, 0.19%</td>
<td>118 ± 9</td>
<td>61 ± 7</td>
<td>233 ± 19</td>
<td>123 ± 8</td>
</tr>
</tbody>
</table>

Cells were harvested and treated for 10 min with the specified agent. The dipyridamole was made up in a stock 10 mM solution in 95% ethanol and stored at -20°C. It was diluted serially in Ringer's solution prior to addition to the cells. The cells were taken for cyclic AMP or tyrosine hydroxylase activity determinations as described in Materials and Methods. The data represent the means ± SEM of triplicate determinations. Similar results were obtained in two other experiments.

*Significantly different from the corresponding control by Student's t test (p < 0.01).
FIG. 1. Time course of DMPP-induced release of ATP from PC12 cells. Harvested PC12 cells in Ringer's solution were dispensed in 58-μl portions in 1.5-ml polyethylene test tubes at ambient temperature. At time zero, a 2-μl portion of 0.3 mM DMPP in Ringer's solution or Ringer's solution alone was added. The cells were centrifuged for 30 s at 13,800 g. The samples required an additional 20 s to decelerate. At the specified time, 50-μl portions of supernatant were added to 15 μl of 0.4 M HClO₄ on ice. The centrifugation was started 1 min prior to the time indicated on the graph. The values are the means of triplicate determinations with an SEM of 15% or less of the value given. Similar results were obtained in five other experiments. ○, 10 μM DMPP; □, control. *Significantly different from control by Student’s t test (p < 0.01).

Metabolism of radioactive ATP, ADP, and AMP by ectoenzymes of PC12 cells

The metabolism of ATP added to intact PC12 cells was studied to determine whether it is metabolized to a form which activates the A₂ adenosine receptor (AMP or adenosine). With an initial concentration of 100 μM, more than 55% was degraded within 1 min (Fig. 2). There was a steady decline in ATP during the 20-min incubation. A maximal accumulation of ADP occurred within 2 min, and its concentration then decreased. After a lag, the amount of AMP/adenosine increased linearly for 5 min and then continued to increase at a reduced rate (Fig. 2). The solvent system used in this analysis failed to resolve AMP and adenosine. The time course of degradation of 100 μM [14C]ADP or [14C]AMP was determined in a parallel incubation. The rate of degradation of AMP was an order of magnitude less rapid than that of ADP (Fig. 3). The rate of degradation of ADP was somewhat less than that of ATP. [14C]ADP loss was accompanied by the concomitant formation of [14C]AMP/AMP. [14C]AMP loss was accompanied by the formation of [14C]adenosine as determined by TLC.

![FIG. 2. Time course of the metabolism of exogenous ATP by intact PC12 cells.](image)

![FIG. 3. Time course of ADP and AMP degradation by PC12 cell suspensions. The experiment was performed as described in Fig. 2 with the following changes: The nucleotide was either [14C]ADP or [14C]AMP at a concentration of 100 μM at zero time, and the radioactivity in the corresponding zone of the chromatogram is shown. The solvent used to develop the chromatogram in the [14C]AMP experiments was 25 mM potassium phosphate (pH 2.5) and 100 mM NaCl. This resolved AMP from the cyclic AMP, adenosine, and ADP. The data are from a representative experiment. Similar results were obtained in three other experiments.](image)

![FIG. 4. Characterization of the release of ATP from PC12 cells.](image)

![TABLE 4. Characterization of the release of ATP from PC12 cells](image)

<table>
<thead>
<tr>
<th>Addition</th>
<th>Extracellular ATP concentration (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>154 ± 18</td>
</tr>
<tr>
<td>DMPP, 10 μM</td>
<td>438 ± 31a</td>
</tr>
<tr>
<td>Hexamethonium, 2 mM</td>
<td>173 ± 15</td>
</tr>
<tr>
<td>DMPP, 10 μM, plus hexamethonium, 2 mM</td>
<td>241 ± 19b</td>
</tr>
<tr>
<td>LaCl₃, 1 mM, no CaCl₂</td>
<td>144 ± 19</td>
</tr>
<tr>
<td>DMPP, 10 μM, plus LaCl₃, 1 mM, no CaCl₂</td>
<td>171 ± 14</td>
</tr>
</tbody>
</table>

Release was measured as described in Materials and Methods. The data represent the means ± SEM of quadruplicate determinations from a single experiment. Similar results were obtained in two other experiments.

* Significantly different from control (p < 0.01) and DMPP plus hexamethionium sample (p < 0.05) by analysis of variance.

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DISCUSSION

The relative effectiveness of exogenous adenine nucleotides in increasing tyrosine hydroxylase activity in PC12 cells was ATP = ADP = AMP > adenosine. This does not agree with the notion that adenosine or AMP preferentially interacts with \( P_1 \) (A1 or A2 subclasses) receptors when compared to ADP or ATP (Burnstock, 1978; Williams, 1987). This order of potency is explained by the observations that ATP and ADP were converted rapidly to AMP and that AMP was converted more slowly to adenosine. Two pieces of evidence point to the activity of 5'-nucleotidase as being a slow step in the metabolism of exogenous ATP by PC12 cells. First, this is illustrated in the time course of degradation of AMP when compared with ADP and ATP (Figs. 2 and 3). Because ATP and ADP inhibit 5'-nucleotidase (Burger and Lowenstein, 1970) and these are AMP precursors, the relative rate of AMP degradation might be even slower than indicated in these experiments. Second, AMP deaminase was more effective in decreasing basal cyclic AMP levels and tyrosine hydroxylase activity than was adenosine deaminase (Table 2).

Our experiments showed that activation of the adenosine receptor lead to an activation of tyrosine hydroxylase through the cyclic AMP second messenger system. We showed that agonists elevated cyclic AMP levels and increased the cyclic AMP-dependent protein kinase activity ratio. In contrast, theophylline, AMP deaminase, and adenosine deaminase treatment decreased these two parameters. Stimulatory and not inhibitory interventions altered cyclic AMP levels and tyrosine hydroxylase activity in PC18 cells. The activation by NECA and 2-chloroadenosine demonstrates that the PC18 cells possessed an adenosine receptor. The lack of an effect of theophylline might be due to a failure of these cells to release adenine nucleotides under basal conditions or to a robust system for inactivating the adenine nucleotides. That the latter was the case was demonstrated by the ability of dipyridamole, an adenosine uptake inhibitor, to bring about the activation of tyrosine hydroxylase in the PC18 cells. These experiments and those with the PC12 cells suggest that the actions of adenosine were terminated by uptake. The failure to observe an effect with deoxycoformycin suggests that endogenous adenosine deaminase was not a physiologically important mechanism for terminating the action of extracellular adenosine.

We demonstrated that DMPP, a nicotinic receptor agonist, evoked the release of ATP in a calcium-dependent fashion. The response was abrogated by hexamethonium (a nicotinic receptor blocker). The ATP might be co-released with catecholamines, acetylcholine, or other transmitter agents found in PC12 cells (Greene and Tischler, 1982). Although ATP might be released independently (Snyder, 1985), Wagner (1985) showed that vesicles isolated from PC12 cells contain dopamine and ATP (at a ratio of 16.5:1). The data presented in Fig. 4 of Wagner (1985) show that potassium depolarization for 1 h greatly decreases vesicular dopamine and ATP. It is probable that at least a portion of ATP release elicited by DMPP in our experiments was derived from this population of vesicles. The measurement of extracellular ATP in these experiments gives only a qualitative estimate of release, because the ATP was undergoing constant degradation. The potency of the ATPase, ADPase, and 5'-nucleotidase in PC12 cells differed from that of smooth muscle cells in culture where all three exhibit comparable activity (Pearson et al., 1980). The relative potency of the three enzymes, however, was similar to that of vascular endothelial cells in culture where 5'-nucleotidase activity is substantially less than that of the other two enzymes (Pearson et al., 1980). We found that the 5'-nucleotidase activity in PC18 cells was comparable to that of the ATPase and ADPase activity (not shown). The lack of an effect of theophylline, adenosine deaminase, and AMP deaminase in PC18 cells and the activating effect of dipyridamole seem most likely due to the rapid metabolism of ATP to adenosine and its rapid inactivation by uptake. Our results suggest that PC12 cells possessed and PC18 cells lacked an autocrine activation of the cyclic AMP second messenger system. This may explain, in part, the greater induction of tyrosine hydroxylase mRNA and activity by 8-bromo-cyclic AMP in PC18 cells than in PC12 cells (Tank et al., 1986). This may also explain why the specific activity of tyrosine hydroxylase was greater in PC12 cells than in PC18 cells (Table 2).

Rabe and McGee (1983) characterized the effects of activating adenosine receptors on PC12 cells on the subsequent evoked release of catecholamines. The absolute values of cyclic AMP under basal and stimulatory conditions in our studies were in good agreement with theirs. That the responses in our study were mediated by \( P_1 \) receptors follows and agrees with the characterization of adenosine receptors in PC12 cells by Williams et al. (1987).

The possibility that released ATP is converted to AMP or adenosine extracellularly, which then func-
tions as a neurotransmitter or neuromodulator, adds another dimension to the biosynthesis of neuroactive agents. Catecholamines are released as such, bring about their response following interaction with their receptor, and are inactivated by uptake. Acetylcholine follows the same sequence, except that it is inactivated by extracellular hydrolysis (Snyder, 1985). Following its release, ATP is metabolized extracellularly to produce the active agents (AMP and adenosine). This extracellular generation is a unique aspect of neurotransmitter or neuromodulator metabolism. Adenosine is then inactivated by uptake in the PC12 or PC18 cell systems. We cannot rule out, however, the release of adenosine per se by the PC12 cells. The observation that AMP deaminase decreased cyclic AMP to the same level as that produced by theophylline suggests that any adenosine released was not enough to produce a significant effect. It is possible that AMP deaminase will be of value in deciphering the relative importance of AMP action in neural, cardiovascular, and platelet function.

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