Adenosine increases the activity of tyrosine 3-monoxygenase in intact pheochromocytoma cells. The effect of adenosine is not dependent upon extracellular Ca++, and is not accompanied by an increase in catecholamine secretion from the cells. Adenosine deaminase decreases the basal activity of tyrosine 3-monoxygenase, and almost completely abolishes the activation of this enzyme by adenosine. In cells treated with adenosine deaminase, 2-chloroadenosine causes a 2- to 5-fold increase in tyrosine 3-monoxygenase activity. 2-Chloroadenosine produces half-maximal activation at a concentration of 0.1 \mu M, and maximal activation at 10 \mu M. Incubation of cells with 2-chloroadenosine produces a stable activation of tyrosine 3-monoxygenase, as measured in vitro. Finally, 2-chloroadenosine increases the content of cAMP in pheochromocytoma cells, and increases the incorporation of \textsuperscript{3H}adenosine into cAMP in cells that have been preincubated with \textsuperscript{3H}adenosine. This rise in cAMP presumably activates the tyrosine 3-monoxygenase by 2-chloroadenosine. Adenosine appears to be an endogenous regulator of tyrosine 3-monoxygenase activity in pheochromocytoma cells.

Chromaffin cells in the adrenal medulla store large amounts of ATP together with catecholamines in chromaffin granules (1, 2). Secretory stimuli cause the coordinate release of ATP and of catecholamines from chromaffin cells (3). Although chromaffin cells apparently secrete intact ATP, this ATP is rapidly degraded within the adrenal gland to a mixture of adenosine, adenosine nucleotides, and other products (5, 6). The function of the secreted ATP, or of its breakdown products, has not been determined.

We have been studying the regulation of catecholamine synthesis in cell suspensions prepared from a transplantable rat pheochromocytoma (5). This tumor, which was originally derived from adrenal medulla (6), resembles normal chromaffin tissue both morphologically (7) and biochemically (8). Tyrosine 3-monoxygenase (EC 1.14.16.2) is the rate-limiting enzyme in the pathway of catecholamine synthesis in both pheochromocytoma cells (9) and normal chromaffin cells (10). We have previously reported that cholera toxin increases the content of cAMP and activates tyrosine 3-monoxygenase in pheochromocytoma cells (9, 11). We now report that adenosine and 2-chloroadenosine increase the activity of tyrosine 3-monoxygenase in pheochromocytoma cells, and that 2-chloroadenosine promotes the accumulation of cAMP in these cells. These experiments suggest that adenosine may participate in the regulation of adenylate cyclase activity and of catecholamine synthesis in chromaffin tissue.

**EXPERIMENTAL PROCEDURES**

**Cell Suspensions**—Suspensions of pheochromocytoma cells were prepared by mechanical disruption of the tumors, as previously described (8), and were preincubated for 30 min at 37°C in medium containing 10 \mu M pargyline. This treatment almost completely inactivates amine oxidase (flavin containing) (EC 1.4.3.4) in the cells (5). Following this preincubation, the cells were collected by centrifugation, washed twice, and resuspended in fresh, pargyline-free medium. The protein content of the cell suspensions was measured by the method of Bradford (12), with bovine serum albumin as a standard.

**Dopa Production and Norepinephrine Secretion**—Cells were incubated for 30 min at 37°C in medium supplemented with 100 \mu M L-tyrosine and 100 \mu M broscine (an inhibitor of aromatic L-amino-acid decarboxylase, EC 4.1.1.28). Cells incubated under these conditions produce dopa, and release almost all (approximately 90%) of this dopa into the incubation medium (9). In addition, the cells secrete norepinephrine into the medium. At the end of the incubation, the samples were chilled, and the cells were removed by centrifugation at 4500 \times g for 10 min at 4°C. Aliquots (250 \mu l) of the supernatant solutions (i.e. the incubation media) were added to 25 \mu l of a solution that contained 1.5 M trichloroacetic acid, 100 \mu M EDTA, and 55 \mu M epinephrine. The samples were filtered through a 0.45-\mu m filter, and the protein content of the samples was measured by the method of Lowry et al. (13) with bovine serum albumin as a standard. The catecholamines in these samples were then assayed by liquid chromatography with electrochemical detection, by a modification of the method of Felice et al. (13). The mobile phase used for this chromatography was 100 mm HPO\textsubscript{4}, titrated to pH 2.6 with NaOH, containing 200 \mu M octyl sodium sulfate and 100 \mu M EDTA; in some experiments, this buffer also contained 2 to 4% methanol. This solvent was pumped at a flow rate of 1.2 ml/min over a Bondapak C\textsubscript{18} column (3.9 \times 300 mm, Waters Associates, Milford, MA). Catecholamines eluted from the column were oxidized at a potential of +0.6 V (versus an Ag/AgCl reference electrode) with a model LC-2A amperometric controller (Bioanalytical Systems, Inc.). The current output of the electrochemical detector was recorded with a chart recorder, and the content of dopa and of norepinephrine in the samples was estimated by measurement of the heights of the peaks produced by the oxidation of these compounds. Dopa production and norepinephrine secretion were estimated after subtraction of the amounts of dopa and norepinephrine found in the incubation media of samples that had been maintained at 0°C for 30 min. Because dopa is produced at a constant rate during the 30-min incubation, we have expressed dopa production in picomoles per min per mg of protein. Norepinephrine secretion is expressed in nanomoles per min per mg of protein. All experiments were carried out several times; only the results of typical experiments are presented. In most experiments, incubations were carried out in triplicate. The results of these experiments are expressed as mean ± S.E. In other experiments, incubations were carried out in duplicate;
the results of these experiments are the averages of closely agreeing determinations.

**In Vitro Tyrosine 3-Monoxygenase Assay**—Cells were incubated for 10 min at 37°C in medium containing adenosine deaminase. At the end of the incubation, the samples were chilled, and the cells were collected by centrifugation. Cells were lysed in 0.5 ml of ice-cold buffer containing 0.1 M NaCl, 0.1 M NaEDTA, 5 mM L-DOPA, 10 mM pyrophosphosphate, and 10 mM sodium phosphate, pH 7.0 (14). Supernatant fractions, obtained after centrifugation at 1800 × g for 10 min, were passed over columns of Dowex 50X-8, 100 to 200 mesh (Na+ form) to remove endogenous catecholamines (6). The pellets were washed with an additional 0.5 ml of digitonin solution and centrifuged, and the resulting supernatant solutions were used to wash the Dowex columns. Tyrosine 3-monoxygenase activity in the effluents of the Dowex columns was measured by a modification of the method of Blank and Pike (15). The 500-μl reaction mixtures contained 25 mg L-tyrosine, 75 mM DL-6-methyltetrahydropterin, 12,500 units of catalase, 2 mM FeSO₄, 100 μM broserine, 7.5 mM 2-mercaptoethanol, 0.1 M sodium dimethylglutarate, pH 6.8, and catecholamine-free extracts corresponding to 50 to 100 μg of cell protein. Reactions were carried out for 15 min at 30°C, and were then terminated by chilling and adding 500 μl of 0.5 M Tris-Cl buffer, pH 8.7, containing 5 mM EDTA, 0.1 M NaCl, 0.25 μM epinephrine. Catecholamines were added to 10 μg of acid-washed alumina, eluted with 150 μl of 100 mM HClO₄, and assayed by liquid chromatography with electrochemical detection, as described above. Epinephrine was used as an internal standard to correct for the recovery of dopa. Tyrosine 3-monoxygenase activity was estimated after subtraction of the dopa produced in enzyme-free incubations, and is expressed as picomoles of dopa produced per min per mg of cell protein.

**Accumulation of cAMP**—Cells were incubated for various times at 37°C in 0.5 ml of medium containing adenosine deaminase. Incubations were terminated by the addition of 50 μl of 3 M HCl and the samples were chilled, and the precipitated protein was removed by centrifugation at 10,000 × g for 10 min at 4°C. The supernatant solution (400 μl) was added to 40 μl of a solution that contained 2.5 mM KCl and 1 mM K₂HPO₄, and the samples were centrifuged again. The samples were placed in the supernatant solution measured by liquid chromatography with UV detection by a modification of the method of Krestulovic et al. (16). The mobile phase used in this chromatography was 100 mM potassium phosphate, pH 5.5, containing 8% methanol (v/v). This solvent was pumped at a flow rate of 1.2 ml/min over a C₈ Ultrasphere-Ion Pair column (4.6 × 150 mm, Altex Scientific, Inc.). cAMP elutes from the column at approximately 13 min. The content of cAMP in the samples was estimated from its absorbance, and is expressed in picomoles per mg of protein.

For these experiments, we measured the accumulation of [³H]cAMP by cells that had been preincubated with [³H]adenine, by a modification of the method of Kebabian et al. (17). Cells were incubated for 45 min at 37°C in medium containing adenosine deaminase and 1.83 μM [³H]adenine, 20 μCi/ml. The cells were then collected by centrifugation washed twice with extracellular [³H]adenine, 5 mM sodium sulfate, and resuspended in fresh medium. The amount of radioactivity incorporated into the cells was determined by liquid scintillation counting. The cells were then incubated for an additional 30 min at 37°C in 1 ml of medium containing adenosine deaminase and 0.7 mM Ro 20-1724, a non-xanthine inhibitor of 3′-5′-cyclic nucleotide phosphodiesterase (EC 3.1.4.17). These incubations were terminated by the addition of 430 μl of a solution containing sodium dodecyl sulfate and cAMP, such that the final concentrations of these substances were 2% (w/v) and 1 mM, respectively. The samples were agitation vigorously with Vortex mixer, and then the cAMP in the samples was isolated by sequential chromatography on columns of Dowex 50 and alumina, as described by Salomon et al. (18). The radioactivity incorporated into [³H]cAMP was measured by liquid scintillation counting, and the recovery of cAMP was estimated by measurement of the absorbance of the samples at 259 nm. The amount of radioactive cAMP incorporated, corrected for recovery, was expressed as a percentage of the total radioactivity in the cells.

**Materials**—The standard medium used in these experiments contained 120 mM NaCl, 4.9 mM KCl, 2.2 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 10 mM glucose, 0.1 mM EDTA, and 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.4). Media containing 56 mM K⁺ were prepared by substituting the appropriate amount of KCl for NaCl. In many experiments, media were supplemented with adenosine deaminase (EC 3.5.4.4). In these experiments, the enzyme was present at 0.4 unit/ml during preincubation, wash, and of the experimental period, and at 0.2 unit/ml during the experimental incubation periods. Adenosine deaminase (type III, from calf intestinal mucosa) was purchased from Sigma Chemical Co., St. Louis, MO. The activity of this enzyme preparation was approximately 200 units/mg of protein; 1 unit of enzyme activity was defined as the decrease of 1 pmol of H₂O₂/min at 25°C in an H₂O₂ concentration of 10 mM. DL-6-Methyltetrahydropterin was purchased from Sigma; 10 mM stock solutions in 1 M 2-mercaptoethanol were stored for short periods at 4°C. Alumina (WN-3 neutral activity grade I) was obtained from Sigma and acid washed according to the method of Anson and Saylor (19). The phosphodiesterase inhibitor 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724) was a gift from Dr. W. E. Scott, Hoffman-La Roche, Inc., Nutley, N.J. [²H]Adenine, 24 Ci/mmol, was purchased from American-Searle Co., Arlington Heights, IL, and used without purification. Other chemicals were reagent grade. Glass-distilled water was used throughout.

**RESULTS**

When pheochromocytoma cells are incubated with tyrosine and broserine, they produce dopa and release this compound into the incubation medium. The rate of dopa production is a measure of the activity of tyrosine 3-monoxygenase in intact pheochromocytoma cells (9). Incubation of these cells in medium containing 56 mM K⁺ increases the rate of dopa production (9). This effect of high K⁺ is dependent upon extracellular Ca²⁺, is inhibited by Co²⁺, and is accompanied by a large increase in the secretion of norepinephrine from the cells (Table I). Adenosine, at a concentration of 100 μM, also increases the production of dopa by pheochromocytoma cells (Table I). In contrast to the action of high K⁺, however, this effect of adenosine is independent of extracellular Ca²⁺, is not inhibited by Co²⁺, and is associated with little change in norepinephrine secretion.

In the experiment reported in Table I, the basal rate of dopa production in the presence of Ca²⁺ was 130 pmol/min/mg of protein, and 100 μM adenosine caused a 40% increase in dopa production. In other experiments, the basal rate of dopa production has ranged between 50 and 550 pmol/min/mg of protein, and the stimulation produced by 100 μM adenosine has ranged between 40 and 100% (data not shown). These experiments were not controlled for the possible effect of endogenously produced adenosine on dopa production. To examine the role of endogenous adenosine in the regulation of tyrosine 3-monooxygenase activity, we measured the effect of adenosine deaminase on dopa production. As shown in Table II, adenosine deaminase significantly decreases (by approximately 45%) the basal rate of dopa production, and almost completely abolishes the stimulation of dopa production by exogenous adenosine. These results support the hypothesis that endogenous adenosine participates in the regulation of tyrosine 3-monooxygenase activity in pheochromocytoma cells. Adenosine deaminase does not reduce the increment in dopa production caused by high K⁺. Thus, the activation of tyrosine 3-monooxygenase by high K⁺ is probably not mediated by the release of adenosine from the cells.

We have also examined the effect of 2-chloroadenosine on dopa production in pheochromocytoma cells. 2-Chloroadenosine is not a substrate for adenosine deaminase (20). In the absence of adenosine deaminase, 10 μM 2-chloroadenosine is approximately equipotent with 100 μM adenosine and causes an 80% increase in dopa production (Table II). Adenosine deaminase appears to potentiate the action of 2-chloroadenosine.
Activation of Tyrosine 3-Monooxygenase by Adenosine

TABLE I

Calcium dependence of dopa production and of norepinephrine secretion

After a 30-min preincubation, pheochromocytoma cells were washed and suspended in Ca²⁺-free medium. These cells were then incubated for 30 min in media containing L-tyrosine and brocresine, as well as Ca²⁺, Co²⁺, and other substances as indicated. To avoid precipitation of cobalt salts, the media used in this experiment contained MgCl₂ instead of MgSO₄, and did not contain KH₂PO₄. After the incubation, cells were separated from the medium, and dopa production and norepinephrine secretion were measured as described under "Experimental Procedures." Results shown are means of duplicate determinations, with individual values shown in parentheses.

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Dopa production</th>
<th>Norepinephrine secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 mm Ca²⁺</td>
<td>0 Ca²⁺</td>
</tr>
<tr>
<td>Control</td>
<td>130 (128, 132)</td>
<td>108 (106, 110)</td>
</tr>
<tr>
<td>56 mM K⁺</td>
<td>270 (257, 283)</td>
<td>139 (137, 141)</td>
</tr>
<tr>
<td>Adenosine (100 μM)</td>
<td>185 (181, 189)</td>
<td>188 (184, 192)</td>
</tr>
</tbody>
</table>

TABLE II

Effect of adenosine deaminase on dopa production

Pheochromocytoma cells were incubated for 30 min in medium containing L-tyrosine and brocresine, in addition to the other substances indicated. In incubations with adenosine deaminase, this enzyme was present during both the preincubation and the subsequent 30-min incubation. After this incubation, cells were removed from the media, and dopa production was measured as described. Results shown are means ± S.E. of incubations carried out in triplicate.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Dopa production</th>
<th>100 μM adenosine</th>
<th>56 mM K⁺</th>
<th>10 μM 2-chloroadenosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>199 ± 9</td>
<td>373 ± 8</td>
<td>340 ± 16</td>
<td>366 ± 9</td>
</tr>
<tr>
<td>Adenosine deaminase</td>
<td>110 ± 5</td>
<td>139 ± 5</td>
<td>274 ± 14</td>
<td>500 ± 13</td>
</tr>
</tbody>
</table>

FIG. 1. Activation of tyrosine 3-monooxygenase by 2-chloroadenosine. Pheochromocytoma cells were incubated for 30 min in media containing L-tyrosine, brocresine, various concentrations of 2-chloroadenosine, and adenosine deaminase as indicated. In incubations containing adenosine deaminase, this enzyme was present both during a 30-min preincubation period and during the 30-min incubation. After this incubation, the cells were removed from the incubation medium, and dopa production was measured as described. For cells incubated in the absence of 2-chloroadenosine, the results shown are means ± S.E. of triplicate incubations. Other points represent the means of duplicate incubations; duplicate values agreed to within 12% or less.

TABLE III

Effect of 2-chloroadenosine and other compounds on dopa production

Pheochromocytoma cells were preincubated in media containing adenosine deaminase, and were then incubated for 30 min in media containing L-tyrosine, brocresine, adenosine deaminase, and other compounds as indicated. After this incubation, cells were separated from the incubation medium, and dopa production was measured as described. Results shown are the means of incubations carried out in duplicate; individual values agreed to within 16% or less.

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Dopa production</th>
<th>pmol/min/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>49</td>
<td>167</td>
</tr>
<tr>
<td>2-Chloroadenosine (10 μM)</td>
<td>167</td>
<td>63</td>
</tr>
<tr>
<td>Guanosine (100 μM)</td>
<td></td>
<td>60</td>
</tr>
<tr>
<td>Inosine (100 μM)</td>
<td></td>
<td>47</td>
</tr>
<tr>
<td>Adenine (100 μM)</td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>Ribose (100 μM)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
inosine stimulate dopa production by 20 to 25%, whereas both adenosine and ribose are inactive. The stimulation of dopa production by inosine may account for the small effect of adenosine on dopa production in adenosine deaminase-treated cells (Table III).

The stimulation of dopa production in intact pheochromocytoma cells by cholera toxin and by 56 mM K⁺ is accompanied by a stable activation of tyrosine 3-monooxygenase, as assayed in vitro (11). To determine whether 2-chloroadenosine also causes a stable activation of tyrosine 3-monooxygenase, we measured enzyme activity in extracts prepared from control and from 2-chloroadenosine-treated cells (Table IV). Under our assay conditions, tyrosine 3-monooxygenase activity in extracts from 2-chloroadenosine-treated cells was 60% greater than enzyme activity in extracts of control cells. 2-Chloroadenosine (10 μM) has no direct effect on tyrosine 3-monooxygenase activity in vitro (Table IV).

Tyrosine 3-monooxygenase in pheochromocytoma cells can be activated by a CAMP-dependent mechanism, both in vitro (22) and in intact cells (9, 11). Adenosine and 2-chloroadenosine are known to activate adenylate cyclase and to increase the content of CAMP in many cell types (23), including brain (24) and neuroblastoma cells (25, 26). We, therefore, studied the effect of 2-chloroadenosine on the content of CAMP in pheochromocytoma cells (Fig. 2). The content of CAMP in unstimulated pheochromocytoma cells is approximately 70 pmol/mg of protein. 2-Chloroadenosine produces a large rise in the CAMP content of these cells. The CAMP content increases without a detectable lag period, is maximal between 2 and 10 min after exposure to 2-chloroadenosine, and then declines slowly after that time. The maximal value of CAMP in 2-chloroadenosine-treated cells is approximately 4 times greater than that in unstimulated cells. Cholera toxin also increases the content of CAMP in pheochromocytoma cells (Fig. 2). The CAMP content in cholera toxin-treated cells (approximately 400 pmol/mg of protein) is somewhat greater than is the content of CAMP in 2-chloroadenosine-treated cells.

In other experiments, we measured the effect of 2-chloroadenosine on the accumulation of [3H]cAMP in the cells that had been preincubated with [2-3H]adenine. The results of one such experiment are shown in Table V. In this experiment, carried out in the presence of the cyclic nucleotide phosphodiesterase inhibitor, Ro 20-1724, [3H]cAMP accounts for 0.41% of the radioactivity in cells incubated under control conditions. Both 2-chloroadenosine (10 μM) and cholera toxin (1 μg/ml) increase the accumulation of [3H]cAMP. The effect of cholera toxin (11-fold) is greater than the effect of 2-chloroadenosine (6-fold). Since 2-chloroadenosine increases the accumulation of [3H]cAMP in cells treated with Ro 20-1724, the nucleoside probably activates adenylate cyclase in these cells.

**DISCUSSION**

Both adenosine and 2-chloroadenosine increase the rate of dopa synthesis in intact pheochromocytoma cells. This increase in dopa synthesis is not dependent upon extracellular Ca²⁺ and is not accompanied by a significant increase in catecholamine secretion from the cells. In addition, 2-chloroadenosine increases the content of cAMP and the incorporation of [3H]adenine into [3H]cAMP in pheochromocytoma cells. In all of these respects, the effects of adenosine nucleosides resemble the action of cholera toxin, and differ from the action of high K⁺ (11). Tyrosine 3-monooxygenase purified from this pheochromocytoma can be activated in vitro by a CAMP-

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**TABLE IV**

<table>
<thead>
<tr>
<th>Source of enzyme</th>
<th>Tyrosine-3-monooxygenase activity (pmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control cells</td>
<td>153 ± 4</td>
</tr>
<tr>
<td>2-Chloroadenosine-treated cells</td>
<td>246 ± 14</td>
</tr>
<tr>
<td>Control cells, 10 μM 2-chloroadenosine in assay</td>
<td>159 ± 4</td>
</tr>
</tbody>
</table>

**TABLE V**

<table>
<thead>
<tr>
<th>Additions</th>
<th>Accumulation of [3H]-cAMP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.41 ± 0.03</td>
</tr>
<tr>
<td>2-Chloroadenosine</td>
<td>2.46 ± 0.16</td>
</tr>
<tr>
<td>Cholera toxin</td>
<td>4.49 ± 0.12</td>
</tr>
</tbody>
</table>
dependent protein kinase (21, 22). The activation of tyrosine 3-monoxygenase in intact cells by adenosine and by 2-chloroadenosine may be mediated, at least in part, by a rise in cAMP and by the activation of a cAMP-dependent protein kinase. The observation that 2-chloroadenosine increases accumulation of \(^{32}\)P]cAMP in cells that have been treated with a cyclic nucleotide phosphodiesterase inhibitor suggests that this nucleoside activates adenylate cyclase in these cells. Although we have not yet measured adenylate cyclase in these cells directly, adenosine and adenosine analogues activate this enzyme and elevate cAMP levels in many other tissues (23).

If 2-chloroadenosine promotes a cAMP-mediated phosphorylation of tyrosine 3-monoxygenase, then incubation of pheochromocytoma cells with 2-chloroadenosine should cause a stable activation of this enzyme. In fact, tyrosine 3-monoxygenase activity is increased in extracts of 2-chloroadenosine-treated cells. There is a quantitative difference, however, between the stimulation of dopa synthesis by 2-chloroadenosine in intact cells (100% to 400%), and the increase in tyrosine 3-monoxygenase activity in extracts of 2-chloroadenosine-treated cells (approximately 60%). We do not understand the reason for this discrepancy. It is unlikely that stimulation of dopa synthesis by 2-chloroadenosine can be accounted for by an effect of the nucleoside on tyrosine transport into cells. Under the conditions of our experiments, the rate of tyrosine uptake into pheochromocytoma cells is much greater than the rate of dopa synthesis, and the intracellular tyrosine concentration is sufficient to support maximal rates of dopa production (9). It is possible, however, that the conditions of our \textit{in vitro} tyrosine 3-monoxygenase assay do not adequately reflect the environment of the enzyme in intact cells. Tyrosine 3-monoxygenase in intact cells may be subject to feedback inhibition by catecholamines. If phosphorylation of the enzyme raises the \(K_d\) for catecholamines (as suggested by Ames \textit{et al.} (27)), then the increase in activity due to phosphorylation may be greater in intact cells than in catecholamine-free cell extracts. Tyrosine 3-monoxygenase activity in intact cells may also be altered independently of enzyme phosphorylation, through changes in the concentrations of allosteric modulators or of its pterin cofactor. Finally, the enzyme may have become partially inactivated (perhaps by dephosphorylation) during preparation of cell extracts.

The reduction in dopa synthesis by adenosine deaminase implies that endogenously produced adenosine has a significant effect on dopa synthesis. For this reason, it is difficult to study the effects of exogenous adenosine on pheochromocytoma cells. We have chosen to circumvent this problem, as others have, by studying the actions of 2-chloroadenosine on cells in the presence of adenosine deaminase (28). 2-Chloroadenosine is not a substrate for adenosine deaminase (20), and is probably a poor substrate for accumulation by cells (29). In many tissues, 2-chloroadenosine and adenosine have similar effects (23, 29, 30). The effects of exogenous 2-chloroadenosine presumably reflect the effects of endogenous adenosine.

The tissue used in these studies resembles normal chromaffin tissue in a number of respects (7, 8). Adenosine may also be a regulator of cAMP metabolism and of tyrosine 3-monoxygenase activity in normal chromaffin cells. Cholinergic stimulation causes the release of large amounts of ATP from chromaffin cells (3). This ATP is rapidly hydrolyzed after its release, and adenosine appears to be a major metabolite of ATP in the adrenal medulla (4, 31). Cholinergic stimulation also increases the content of cAMP in the adrenal medulla, but cholinergic agonists do not directly activate adenylate cyclase in this tissue (32). The activation of adenylate cyclase in chromaffin cells by adenosine could provide an attractive explanation for the stimulus-coupled increase in cAMP in these cells.

**REFERENCES**