Histidine Regulation of Cyclic AMP Metabolism in Cultured Renal Epithelial LLC-PK₁ Cells*

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1-Histidine and imidazole (the histidine side chain) significantly increase cAMP accumulation in intact LLC-PK₁ cells. This effect is completely inhibited by isobutylmethylxanthine (IBMX). Histidine and imidazole stimulate cAMP phosphodiesterase activity in soluble and membrane fractions of LLC-PK₁ cells suggesting that the IBMX-sensitive effect of these agents to stimulate cAMP formation is not due to inhibition of cAMP phosphodiesterase. Histidine and imidazole but not alanine (the histidine core structure) increase basal, GTP-, forskolin-, and AVP-stimulated adenylate cyclase activity in LLC-PK₁ membranes. Two other amino acids with charged side chains (aspartic and glutamic acids) increase AVP-stimulated but not basal- nor forskolin-stimulated adenylate cyclase activity. This suggests that multiple amino acids with charged side chains can regulate selected aspects of adenylate cyclase activity. To better define the mechanism of histidine regulation of adenylate cyclase, membranes were detergent-solubilized which prevents histidine and imidazole potentiation of forskolin-stimulated adenylate cyclase activity and suggests that an intact plasma membrane environment is required for potentiation. Neither pertussis toxin nor indomethacin pretreatment alter imidazole potentiation of adenylate cyclase. IBMX pretreatment of LLC-PK₁ membranes also prevents imidazole to potentiate adenylate cyclase activity. Since IBMX inhibits adenylate cyclase coupled adenosine receptors, LLC-PK₁ cells were incubated in vitro with 5'-N-ethylcarboxamidoadenosine (NECA) which produced a homologous pattern of desensitization of NECA to stimulate adenylate cyclase activity. Despite homologous desensitization, histidine and imidazole potentiation of adenylate cyclase was unaltered. These data suggest that histidine, acting via an imidazole ring, potentiates adenylate cyclase activity and thereby increases cAMP formation in cultured LLC-PK₁ epithelial cells. This potentiation requires an intact plasma membrane environment, occurs independent of a pertussis toxin-sensitive substrate and of products of cyclooxygenase, and is inhibited by IBMX. This IBMX-sensitive pathway does not involve either inhibition of cAMP phosphodiesterase activity or a stimulatory adenosine receptor coupled to adenylate cyclase.

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Amino acids play a critical role in maintaining cellular homeostasis by serving as precursors for protein synthesis and substrates for metabolism. Amino acids also appear capable of regulating several aspects of cellular function including ion permeability, intracellular osmolality, and secretory processes (1-3). One potential mechanism whereby amino acids regulate cellular function is through their effects on cyclic nucleotide metabolism. In 1976, Shimizu et al. (4-6) and subsequently Schmidt and co-workers (7, 8) found that glutamic and aspartic acids increase cAMP formation in guinea pig and rat brain slices. The mechanism whereby amino acids regulate cAMP formation was not determined in these studies. Moreover, there is little information on amino acid regulation of cyclic nucleotide metabolism in nonneural tissue and on the effects of other amino acids on regulation of cAMP accumulation.

The posterior pituitary hormone vasopressin (AVP) acts through a V₂ receptor to stimulate the formation of cAMP producing an increase in water permeability in target renal epithelial cells (9). Recently, the amino acid histidine was found to enhance AVP-stimulated water permeability in toad urinary bladder (10). Although these observations suggest the possibility that histidine enhances AVP-stimulated cAMP formation, the biochemical mechanism of histidine potentiation of AVP action was not determined. We therefore studied the effects of histidine on cAMP metabolism in AVP-responsive LLC-PK₁ renal epithelial cells. We find that histidine enhances cAMP accumulation by stimulating adenylate cyclase activity. Comparable stimulation of adenylate cyclase activity can be produced by imidazole (the histidine side chain) but not by alanine (the histidine core). Additional experiments were undertaken to elucidate the mechanism whereby histidine and imidazole stimulate adenylate cyclase activity. Our results suggest that histidine and imidazole stimulate adenylate cyclase activity and cAMP accumulation via a novel plasma membrane-dependent, IBMX-sensitive pathway that does not involve either inhibition of cAMP phosphodiesterase or activation of a stimulatory adenosine receptor coupled to adenylate cyclase.

EXPERIMENTAL PROCEDURES

Materials—Arginine vasopressin, L-histidine, D-histidine, α-methylhistidine, 1-methylhistidine, L-alanine, L-phenylalanine, L-glutamine, L-glutamic acid, L-aspartic acid, indomethacin, isoproterenol, imidazole, and GTP were purchased from Sigma. Forskolin and IBMX were purchased from Calbiochem. 5'-N-Ethylcarboxamidoadenosine was purchased from Research Biochemicals. Pertussis toxin and substrates for metabolism.

The abbreviations used are: AVP, arginine vasopressin; NECA, 5'-N-ethylcarboxamidoadenosine; IBMX, 3-isobutyl-1-methylxanthine; G, G-protein that can function to inhibit adenylate cyclase activity; Hepes, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid.
Historically, to use an ATT PC-6300 desktop computer and ABSTAT software. Immunoassay kits were from Incstar Inc. and Amersham Corp. Membranes obtained from cells grown on 75-cm² plastic flasks while 24-well panels obtained from Du Pont-New England Nuclear and the CAMP radioassay for adenylate cyclase and CAMP phosphodiesterase assays were obtained. Cells were grown to confluence and harvested in RPMI 1640 medium (Irving Scientific) which was supplemented with 200 mg % sodium bicarbonate, 16 mM Hepes, 100 units/ml penicillin, and 100 μg/ml streptomycin. Newborn calf serum (6%) was added to the media for the initial 3-7 days of growth while monolayer proliferation was established, after which all cultures received serum-free media. The cultures were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO₂. Cell growth was monitored under an inverted microscope with phase optics (Nikon IM35) and typically reached confluence in 5-7 days. Confluent cultures were selected for biochemical studies.

**Crude Membrane Preparation**—Confluent cultures were washed three times with 8 ml of cold phosphate-buffered saline containing 0.9 mM CaCl₂ and 0.5 mM MgCl₂ and the cells harvested by scraping 3 times with 4 ml of ice-cold EDTA (1 mM) (Tris (50 mM) buffer (pH 7.4). Membranes were homogenized with 15 strokes of a Dounce homogenizer, centrifuged at 14,000 rpm at 4 °C for 5 min, and resuspended in 500 μl of EDTA/Tris buffer.

**Adenylate Cyclase Assay**—Adenylate cyclase activity was measured using slight modifications of previously described techniques and carried out on a final volume of 50 μl (11-13). Ten μl of membrane preparation (~10 μg of protein) was added to an assay mixture containing 0.25 mM Na₂EDTA, 5.25 mM MgCl₂, 1.0 mM cAMP, 1.0 mM purified [α-³²P]ATP (2 x 10⁶ cpm) in 25 mM Tris-HCl (pH 7.4) and an ATP-regenerating system consisting of 20 mM creatine phosphate and 100 IU/ml creatine phosphokinase. Because Tris-HCl does not possess maximal buffering capacity at pH 7.4, some experiments at pH 7.4 were performed using 25 mM Hepes (pH 7.5) as the primary buffer. After a 10-min incubation at 30 °C, the reaction was stopped by adding 150 μl of ice-cold stopping solution containing 3.3 mM ATP, 50 mM Tris-HCl, 5.0-100-150 mM of [³²P]cAMP for recovery which averaged 50-70%. [³²P]cAMP was separated from unreacted [α-³²P]ATP by a sequential two-step elution over Dowex and alumina columns. Assays were carried out in triplicate or quadruplicate with a standard error of the mean usually less than 2-3%.

In some experiments, aliquots of membranes were solubilized by mixing them with buffer containing (final concentration), 0.2-1% Lubrol FX, 20 mM Tris-HCl (pH 7.5), 1 mM MgCl₂, 1 mM EDTA, 250 mM NaCl, incubating them with 1°C for 60 min and centrifuging them at 100,000 x g for 1 h at 4 °C. The supernatant was decanted and analyzed for adenylate cyclase as described above.

**cAMP Phosphodiesterase Activity Assay**—Phosphodiesterase activity was measured by a slight modification of the method described by Thompson et al. (14). Briefly, 10 μl of either cytosol or membranes were added to buffer (final concentration 40 mM Tris-HCl and 2.0 mM MgCl₂ (pH 8.0)), and the reaction initiated with either 0.1 mM cAMP for measurements of total activity or 0.5 μM cAMP for measurements of high affinity activity. Incubation time was 20 min at 30 °C and stopped by placing tubes in boiling water for 45 s. Following treatment with King Cobra snake venom (10 min, 30 °C), the samples were applied to Dowex AG 1-X5 (200-400 mesh) columns and the [³²P]cAMP product eluted with 1.5 ml of methanol and quantitated by liquid scintillation counting. Blank values averaged less than 2-3% of total counts. Membranes for cAMP phosphodiesterase activity were prepared as described above. For studies in soluble fraction, membranes were removed by centrifugation at 32,500 rpm (100,000 x g) for 30 min.

**cAMP Assay**—Cells were grown to confluence in 24-well plates and kept serum-free for 24 h prior to assay. Assays were performed in physiologic saline solution (in mM) NaCl 145, KCl 5.0, MgCl₂ 1.0, CaCl₂ 1.0, glucose 10, Hepes 20 (pH 7.5) with Tris containing the indicated effectors agents and incubated for 12 min at 30 °C. Extracellular cAMP was quantitated by radioimmunoassay using kits from either Incstar Inc. or Amersham Corp.

**Protein Assay**—Protein was estimated by a modified Lowry method (15) using bovine serum albumin as a standard.

**Statistical Analyses**—All calculations and analyses were carried out using an IBM PC-G6000 desktop computer and ABSTAT software. All data are expressed as the mean ± S.E. Statistical analyses were performed using paired and unpaired Student's t test and ANOVA where appropriate. A p value < 0.05 is considered significant.

**RESULTS**

Recent physiologic experiments indicate that histidine increases water transport in an AVP-responsive epithelium (10). To determine if histidine's effect on water transport could be mediated by cAMP, we measured the effect of histidine on CAMP levels in LLC-PK₁ cells (Table I). The results show that L-histidine and imidazole, histidine side chain, increase CAMP and this effect is blocked by pretreatment with 1.0 mM IBMX. For example, cAMP accumulation (picomoles/mg/12 min) under basal, IBMX, histidine, and IBMX + histidine conditions was 0.57, 1.57, 1.97, and 1.35, respectively. Addition of either forskolin (10⁻⁵ M) or vasopressin (10⁻⁴ M) results in a 3-5-fold increase in CAMP accumulation in the presence of IBMX (data not shown). This suggests that an effect of IBMX to prevent histidine and imidazole to increase CAMP accumulation is not due to maximal stimulation of CAMP formation.

Further studies were performed to delineate the IBMX-sensitive mechanism whereby histidine and imidazole increase CAMP accumulation in LLC-PK₁ cells. One of the well-known effects of IBMX is inhibition of cAMP phosphodiesterase activity. To assess the possibility that histidine and imidazole inhibit cAMP phosphodiesterase activity, the effect of these agents on the activity of soluble and particulate forms of cAMP phosphodiesterase was measured (Table II). Although we have not completely characterized the specific types of cAMP phosphodiesterase present in these cells, we have determined that 10⁻⁶ M cAMP does not affect either total or high affinity enzyme activity in particulate fraction. cGMP does however increase high affinity enzyme activity by 46% in cytosolic fraction while not altering total cytosolic enzyme activity (data not shown). Both L-histidine and imidazole stimulate total and high affinity cAMP phosphodiesterase activity in soluble and particulate fraction, while the control, IBMX, consistently produces inhibition. These observations do not exclude the possibility that histidine and imidazole inhibit a specific type of cAMP phosphodiesterase. However, these results do suggest that the overall effect of imidazole to stimulate LLC-PK₁ cell CAMP accumulation is not due to inhibition of cAMP phosphodiesterase activity.

We next examined the effect of various concentrations of L-histidine on basal, AVP-, forskolin-, and GTP-stimulated adenylate cyclase activity in crude LLC-PK₁ membranes (Fig. 1). At 0.5 mM, L-histidine inhibits AVP- and forskolin-stimulated adenylate cyclase activity while at 25 mM, L-histidine significantly potentiates basal, GTP-, forskolin-, and AVP-stimulated adenylate cyclase activity. At 25 mM, histidine and imidazole result in consistent potentiation of submaximal and maximal concentration.
Histidine Stimulation of Adenylate Cyclase

TABLE II
Effect of L-histidine, imidazole, and IBMX on cAMP phosphodiesterase activity in LLC-PK₁ membranes and cytosol

LLC-PK₁ membranes and soluble fractions were prepared as described under “Experimental Procedures.” When present in the assay, the concentration of IBMX was 1 mM and L-histidine and imidazole were 25 mM. Total activity was measured with a cAMP substrate concentration of 0.1 mM while high affinity activity with a 0.5 μM concentration of cAMP substrate. All values were expressed as the mean ± S.E. of duplicate determinations performed on 3–4 separate assays.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Membrane Total</th>
<th>High affinity</th>
<th>Soluble Total</th>
<th>High affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.22 ± 0.29</td>
<td>0.263 ± 0.07</td>
<td>38.3 ± 4.0</td>
<td>1.29 ± 0.08</td>
</tr>
<tr>
<td>IBMX</td>
<td>1.17 ± 0.15</td>
<td>0.033 ± 0.01</td>
<td>5.93 ± 2.4</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>Histidine</td>
<td>3.84 ± 0.41</td>
<td>0.295 ± 0.07</td>
<td>46.9 ± 3.4</td>
<td>1.12 ± 0.07</td>
</tr>
<tr>
<td>Imidazole</td>
<td>5.44 ± 0.63</td>
<td>0.303 ± 0.06</td>
<td>55.1 ± 6.6</td>
<td>1.46 ± 0.03</td>
</tr>
</tbody>
</table>

*p < 0.05 when compared with control.

![Graph](http://example.com/graph1.png)

FIG. 1. Effect of varying concentrations of L-histidine on basal, GTP-, forskolin-, and AVP-stimulated adenylate cyclase activity in LLC-PK₁ membranes. Membranes were prepared as described under “Experimental Procedures.” When present in the assay, the concentration of GTP was 10 μM, forskolin 50 μM, and AVP 10 nM. Values shown are the mean ± S.E. of triplicate experiments.

![Graph](http://example.com/graph2.png)

FIG. 2. Effect of L-histidine on AVP- and forskolin-stimulated adenylate cyclase activity in LLC-PK₁ membranes. Membranes were prepared as described under “Experimental Procedures.” When present in the assay, the concentration of L-histidine was 25 mM. All values represent ± S.E. of triplicate determinations done in a paired fashion on three separate membrane preparations.

Additional experiments were undertaken to determine the mechanism of histidine and imidazole to stimulate adenylate cyclase activity. Since the Tris buffer used in most assays does not possess maximal capacity at pH 7.4 and since imidazole may exert pH buffering effects, studies were performed with Hepes as the assay buffer (Table III). In the presence of Hepes buffer, imidazole significantly potentiates forskolin- and AVP-stimulated adenylate cyclase activity at 0.5 mM concentration. To determine if imidazole alters the separation of unreacted ATP from CAMP during chromatography, we measured the elution profile of ATP and CAMP from both the Dowex and alumina columns in the presence and absence of 25 mM imidazole and found no effect of imidazole. In addition, imidazole did not affect final recovery of CAMP from the alumina column and blank values were the same in the presence and absence of imidazole suggesting no significant contamination with any unreacted ATP.

The molecular features responsible for histidine potentiation of AVP-stimulated adenylate cyclase activity were examined (Table IV). Potentiation of AVP-stimulated adenylate cyclase activity occurred with the imidazole side chain of histidine but not with alanine, the histidine core structure. Potentiation of AVP-stimulated adenylate cyclase activity also occurred with L-histidine, the nonmetabolized stereoisomer of L-histidine, and with L-histidine derivatives that were methylated on either the side chain or the core structure. To determine if the effect of histidine to promote adenylate cyclase activity is unique to this amino acid, we measured the effect of other amino acids with varying side chain characteristics on AVP-stimulated adenylate cyclase activity (Table IV). Neither phenylalanine (another amino acid which, like...
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Imidazole and histidine have been shown to inhibit prostaglandin and thromboxane biosynthesis in AVP-responsive tissues (16, 17). Prostaglandins and thromboxanes are well known to exert regulatory influences on adenylate cyclase activity in AVP-responsive cells (16, 17). We therefore examined the effect of cyclooxygenase inhibition with large concentrations of indomethacin which have been shown to abolish production of cyclooxygenase products in cultured renal epithelial cells (18), on imidazole potentiation of adenylate cyclase activity (Table VI). Imidazole potentiated basal and forskolin-stimulated adenylate cyclase activity equivalently in the presence and absence of indomethacin suggesting that imidazole potentiation of enzyme activity can occur independent of products of cyclooxygenase.

To determine if IBMX affects imidazole potentiation of adenylate cyclase activity, the effect of 25 mM imidazole to potentiate 50 μM forskolin-stimulated adenylate cyclase activity was measured in the presence and absence of 1 mM IBMX. In the absence of IBMX, imidazole increased forskolin-stimulated adenylate cyclase activity by 53% (from 178.3 ± 12.4 to 273.6 ± 27.3 pmol/mg/min) while in the presence of IBMX, the same concentration of imidazole increased forskolin-stimulated adenylate cyclase activity by 7% (from 211.4 ± 19.6 to 230.1 ± 18.7 pmol/mg/min).

In addition to inhibiting cAMP phosphodiesterase activity, IBMX also is known to act as an adenosine receptor antagonist (19). Two cell surface adenosine receptors are known to be linked to adenylate cyclase activity, one in an inhibitory and the other in a stimulatory fashion (20–25). Cell surface adenosine receptors that inhibit adenylate cyclase activity have been demonstrated to be present in LLC-PK₁ membranes (23) but, to our knowledge, adenosine receptors that stimulate adenylate cyclase activity have not been demonstrated in LLC-PK₁ cells. Therefore, we examined the effect of NECa, a known agonist for adenosine receptors that stimulate adenylate cyclase activity, on enzyme activity (Table VII). Treatment with NECa produces a dose-dependent increase in adenylate cyclase activity. The effect of 10 μM NECa to stimulate adenylate cyclase activity was completely inhibited by 1 mM IBMX (data not shown). Together, these observations support the presence of IBMX-inhibitable adenosine receptor that stimulates adenylate cyclase activity in these LLC-PK₁ cells.

To delineate if histidine and imidazole potentiation of adenylate cyclase activity is mediated via a stimulatory adenosine receptor, we desensitized cells to the stimulatory aden-
and the concentration of AVP was 10 nM. Glutamine, L-aspartic acid, L-glutamic acid, and imidazole was 25 mM. When present in the assay, the concentration of GTP and other selected amino acids on AVP-stimulated adenylate cyclase activity in LLC-PK₁ membranes

LLC-PK₁ membranes were prepared and assayed for adenylate cyclase activity as described under "Experimental Procedures." When present in the assay, the concentration of L- and D-histidine, L-methylhistidine, α-methyl-histidine, L-alanine, L-phenylalanine, L-glutamine, L-aspartic acid, L-glutamic acid, and imidazole was 25 mM and the concentration of AVP was 10 nM. The data represent the mean ± S.E. for triplicate determinations performed in three separate experiments. All values are significantly different (p < 0.05) than no treatment except with alanine, phenylalanine, and glutamine treatment.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Adenylate cyclase activity (pmol/mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.0 mM</td>
</tr>
<tr>
<td>Basal</td>
<td>9.0 ± 1.5</td>
</tr>
<tr>
<td>Forskolin (10⁻⁵ M)</td>
<td>174.1 ± 28.8</td>
</tr>
<tr>
<td>Arginine vasopressin (10⁻⁵ M)</td>
<td>78.4 ± 5.8</td>
</tr>
</tbody>
</table>

*p < 0.05 when compared with control.

Table III

Effect of histidine, histidine analogues, imidazole, and other selected amino acids on AVP-stimulated adenylate cyclase activity in LLC-PK₁ membranes

LLC-PK₁ cell membranes were exposed to 1 pg/ml pertussis toxin for 18 h. Membranes were prepared and assayed as described under "Experimental Procedures." When present in the assay, the concentration of imidazole was 25 mM. The data are the mean ± S.E. of triplicate determinations performed on three separate membrane preparations.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Adenylate cyclase activity (pmol/mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5.1 ± 1.4</td>
</tr>
<tr>
<td>Imidazole (−)</td>
<td>15.9 ± 1.9</td>
</tr>
<tr>
<td>Imidazole (+)</td>
<td>47.3 ± 9.6</td>
</tr>
<tr>
<td>AVP (10 nM)</td>
<td>91.2 ± 5.3</td>
</tr>
<tr>
<td>Forskolin (10 µM)</td>
<td>117.3 ± 5.1</td>
</tr>
</tbody>
</table>

Table IV

Modulation of adenylate cyclase activity by imidazole in LLC-PK₁ membranes incubated in the presence and absence of pertussis toxin

LLC-PK₁ cell membranes were incubated in the presence and absence of indomethacin. Membranes were prepared and assayed as described under "Experimental Procedures." When present in the assay, the concentration of indomethacin was 10 µM, forskolin 50 µM, and imidazole 25 mM. The data are the mean ± S.E. of triplicate determinations on three separate membrane preparations.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Adenylate cyclase activity (pmol/mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>8.7 ± 0.4</td>
</tr>
<tr>
<td>Imidazole (−)</td>
<td>11.8 ± 0.8</td>
</tr>
<tr>
<td>Forskolin</td>
<td>200.5 ± 8.2</td>
</tr>
<tr>
<td>Imidazole (+)</td>
<td>272.1 ± 27.9</td>
</tr>
</tbody>
</table>

Table V

Modulation of adenylate cyclase activity by imidazole in LLC-PK₁ membranes incubated in the presence and absence of indomethacin

Fig. 4. Effect of aspartic (closed symbols) and glutamic (open symbols) acids on basal, GTP-, forskolin-, and AVP-stimulated adenylate cyclase activity in LLC-PK₁ membranes. Membranes were prepared as described under "Experimental Procedures." When present in the assay, the concentration of GTP was 10 µM, forskolin 50 µM, and AVP 10 nM. Values shown are the mean of triplicate determinations done on three to four membrane preparations. Significant potentiation of AVP-stimulated enzyme activity occurred at all concentrations of aspartic acid and at 5.0 and 25.0 mM glutamic acid.

Histidine Stimulation of Adenylate Cyclase
TABLE VII

Modulation of adenylate cyclase activity by NECA pretreatment in LLC-PK1 membranes

<table>
<thead>
<tr>
<th>Condition</th>
<th>Adenylate cyclase activity (pmol/mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>Control: 5.6 ± 0.0, NECA: 5.9 ± 0.7</td>
</tr>
<tr>
<td>NECA</td>
<td>7.1 ± 0.0</td>
</tr>
<tr>
<td>10^-7 M</td>
<td>11.1 ± 1.0</td>
</tr>
<tr>
<td>10^-6 M</td>
<td>16.7 ± 1.9</td>
</tr>
<tr>
<td>AVP</td>
<td>93.7 ± 8.5</td>
</tr>
<tr>
<td>+L-histidine</td>
<td>125.7 ± 5.9</td>
</tr>
<tr>
<td>+imidazole</td>
<td>186.3 ± 14.9</td>
</tr>
<tr>
<td>Forskolin</td>
<td>214.6 ± 12.1</td>
</tr>
<tr>
<td>+L-histidine</td>
<td>267.1 ± 17.1</td>
</tr>
<tr>
<td>+imidazole</td>
<td>443.2 ± 43.9</td>
</tr>
</tbody>
</table>

*p < 0.05 when compared to controls.

DISCUSSION

In present studies, we found that selected amino acids with charged side chains such as histidine and glutamic and aspartic acids potentiated AVP-stimulated adenylate cyclase activity in LLC-PK1 membranes. Such an effect was not seen with uncharged amino acids such as alanine, phenylalanine, and glutamine. To our knowledge, an effect of an amino acid to regulate adenylate cyclase activity has not been previously demonstrated. Shimizu et al. (4-6) and Schmidt and co-workers (7, 8) reported that glutamic and aspartic acids increase brain tissue cAMP content. However, those workers did not find an effect of glutamic and aspartic acids on either adenylate cyclase or cAMP phosphodiesterase activity (4-8).

The mechanism and site whereby selected amino acids regulate adenylate cyclase activity likely differs. For example, we found that histidine potentiates basal, GTP-, forskolin-, and AVP-stimulated enzyme activity suggesting a prominent distal site of action, perhaps at the catalytic subunit. By contrast, glutamic and aspartic acids potentiate only AVP-stimulated adenylate cyclase activity suggesting a receptor or receptor-G-protein coupling site of action.

Histidine potentiation of AVP-stimulated adenylate cyclase did not occur with alanine but was consistently seen with imidazole, the histidine side chain. Our results suggest that imidazole does not stimulate adenylate cyclase activity by altering assay conditions such as by acting as a pH buffer or by affecting substrate availability. We found that imidazole also potentiates adenylate cyclase activity in MDCK cell membranes. Solubilization of the crude membrane preparation resulted in loss of imidazole and L-histidine potentiation of forskolin-stimulated adenylate cyclase activity while such potentiation continued to occur in the presence of large concentrations of pertussis toxin and indomethacin. Together, these observations suggest that imidazole potentiation of adenylate cyclase is not tissue-specific and requires an intact plasma membrane environment. The effects of imidazole might involve the interaction between G proteins and the catalytic unit of adenylate cyclase, but failure of pretreatment with pertussis toxin to reverse these effects suggests that the effects of imidazole are not mediated by G. It appears that imidazole potentiation can occur independent of products of cyclooxygenase.

The effect of histidine and imidazole to increase adenylate cyclase activity in LLC-PK1 membranes and cAMP content in intact LLC-PK1 cells could be blocked by IBMX. It is noteworthy that the effects of glutamic and aspartic acids to stimulate cAMP formation in brain tissue are also attenuated by IBMX (4-8). IBMX is known to inhibit cAMP phosphodiesterase activity and soluble and particulate forms of this enzyme are clearly present in cultured renal epithelial cells (26). An effect of imidazole to both stimulate (27, 28) and inhibit (27) cAMP phosphodiesterase activity has been reported previously. In our studies, histidine and imidazole stimulate rather than inhibit total and high affinity forms of cAMP phosphodiesterase activity. These observations suggest that histidine and imidazole potentiation of adenylate cyclase activity and enhancement of cAMP accumulation cannot be explained on the basis of cAMP phosphodiesterase inhibition. Our observations do provide, however, documentation that amino acids may act at more than one site to regulate cellular cAMP content.

Another well characterized effect of IBMX is antagonism of adenosine receptors (19). An adenosine receptor coupled to adenylate cyclase in a stimulatory fashion, that can be blocked by IBMX, has been demonstrated in many tissues (20-23). We also found an IBMX-sensitive adenosine pathway that stimulates adenylate cyclase activity in LLC-PK1, cell membranes. To determine if L-histidine and imidazole act via this adenosine receptor to stimulate adenylate cyclase, we desensitized cells by exposure to NECA. Cells pretreated with NECA demonstrated a significant 50-60% homologous desensitization of NECA stimulated adenylate cyclase activity. Despite significant desensitization of LLC-PK1, membranes, the effects of both L-histidine and imidazole to potentiate AVP- and forskolin-stimulated adenylate cyclase activity in these membranes remained unchanged. Together, these observations suggest that L-histidine and imidazole potentiate adenylate cyclase activity by an IBMX inhibitable pathway that does not involve a stimulatory adenosine receptor. It is noteworthy that IBMX may regulate cellular calcium flux and GTPase activity in other cells, notably myocytes (29, 30).

Further studies will, however, be necessary to delineate the mechanism of IBMX inhibition of histidine and imidazole potentiation of adenylate cyclase activity.

In summary, our studies suggest that histidine, acting through its imidazole ring, can stimulate adenylate cyclase activity and cAMP formation in renal epithelial cells. The effect of histidine to promote AVP-stimulated adenylate cyclase activity is not unique to this amino acid since a similar effect is seen with other amino acids such as aspartic and glutamic acids that contain charged side chains. The effect of L-histidine and imidazole to potentiate adenylate cyclase activity appears to require an intact plasma membrane environment and to occur independent of a pertussis toxin-sensitive G protein and products of cyclooxygenase. This pathway can be blocked by IBMX. This IBMX-sensitive pathway does not appear to involve either inhibition of cAMP phosphodiesterase activity or a stimulatory adenosine receptor coupled to adenylate cyclase. Further studies are necessary to clarify the IBMX-sensitive mechanism(s) whereby L-histidine and imidazole stimulate adenylate cyclase activity.

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