Modulation of the cyclic AMP content of rat renal inner medulla by oxygen: possible role of local prostaglandins.

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Research Article

The lower O2 tension and more active anerobic metabolism that pertain in the inner medulla (IM) of kidney relative to cortex (C) are well recognized, but there is no evidence that O2 availability constitutes a limiting or regulatory factor in IM metabolism or function. In the present in vitro study, we examined the effects of O2 on adenosine 3',5'-monophosphate (cAMP) metabolism in slices of rat renal C and IM. After a 20-min incubation of slices in Krebs Ringer bicarbonate buffer with 95% O2 + 5% CO2 serving as the gas phase, the cAMP content of IM was 6-10 fold higher than that of C in either the presence or absence of 2 mM 1-methyl-3-isobutylxanthine in the incubation media. In slices of IM incubated for 20 min with 1-methyl-3-isobutylxanthine, cAMP was 22.5+/-SE 2.48 pmol/mg wet weight at 95% O2 and 4.37 without O2. Oxygenation of O2-deprived IM increased cAMP twofold in 2 min, an effect fully expressed in 5 min (fivefold increase). Further, cAMP of IM rose progressively and significantly over a range of atmospheric O2 content from 0 to 50% conditions which should reproduce and encompass O2 tensions that pertain in tissues in vivo. By contrast, basal cAMP content of C varied less than twofold in the presence of 95% versus no O2, implying that O2 modulation of cAMP was […]

Modulation of the Cyclic AMP Content of Rat Renal Inner Medulla by Oxygen

POSSIBLE ROLE OF LOCAL PROSTAGLANDINS

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ABSTRACT The lower O_2 tension and more active anerobic metabolism that pertain in the inner medulla (IM) of kidney relative to cortex (C) are well recognized, but there is no evidence that O_2 availability constitutes a limiting or regulatory factor in IM metabolism or function. In the present in vitro study, we examined the effects of O_2 on adenosine 3',5'-monophosphate (cAMP) metabolism in slices of rat renal C and IM. After a 20-min incubation of slices in Krebs Ringer bicarbonate buffer with 95% O_2 + 5% CO_2 serving as the gas phase, the cAMP content of IM was 6–10-fold higher than that of C in either the presence or absence of 2 mM 1-methyl-3-isobutylxanthine in the incubation media. In slices of IM incubated for 20 min with 1-methyl-3-isobutylxanthine, cAMP was 22.5±SE 2.48 pmol/mg wet weight at 95% O_2 and 4.37 without O_2. Oxygenation of O_2-deprived IM increased cAMP twofold in 2 min, an effect fully expressed in 5 min (fivefold increase). Further, cAMP of IM rose progressively and significantly over a range of atmospheric O_2 content from 0 to 50%, conditions which should reproduce and encompass O_2 tensions that pertain in tissues in vivo. By contrast, basal cAMP content of C varied less than twofold in the presence of 95% versus no O_2, implying that O_2 modulation of cAMP was specific for IM.

Indomethacin and meclofenamate, structurally distinct inhibitors of prostaglandin synthesis, both significantly decreased basal cAMP accumulation in oxygenated slices of IM but not of C. Meclofenamate also reduced basal adenylate cyclase activity determined in homogenates prepared from slices of IM which had been incubated at 95% O_2. In slices of IM previously exposed to indomethacin or meclofenamate at 95% O_2, a maximally effective concentration of exogenous prostaglandin E_1 restored cAMP and adenylate cyclase activity to levels which approximated those observed at 95% O_2 in the absence of an inhibitor of prostaglandin synthesis. These results suggest that O_2 enhancement of cAMP content in IM may be mediated at least in part by local prostaglandins.

INTRODUCTION

The unique anatomical and physiological properties of the renal inner medulla appear to be correlated with distinctive metabolic characteristics. The content of mitochondria and of several enzyme systems involved in oxidative metabolism are greatly reduced in this tissue (1–4), while anaerobic glycolysis is much more active than in other areas of the kidney (5, 6). Moreover, several in vivo observations indicate that the inner medulla is ordinarily exposed to oxygen tensions of 30–50 mm Hg, levels significantly below those that normally exist in the cortex (7–9). Total O_2 delivery to, and consumption by, the inner medulla have also been estimated to be only 3–5% of cortical values (10). Despite the low O_2 tensions that pertain in inner medulla, there is no evidence that O_2 availability is a limiting or regulatory factor in the metabolism or function of this tissue (9). The latter possibility was initially suggested to us by the finding that cyclic adenosine 3',5'-monophosphate (cAMP)[1] levels of slices of inner

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[1] *Abbreviations used in this paper*: cAMP, adenosine 3',5'-monophosphate; MIX, 1-methyl-3-isobutylxanthine; PTH, parathyroid hormone; PGE_1, prostaglandin E_1; AVP, arginine vasopressin; KRBG, Krebs Ringer bicarbonate containing 1 mg/ml of glucose and albumin with 95% O_2 and 5% CO_2 serving as the gas phase.

medulla exposed to high oxygen tension were consistently 6–10-fold higher than those of cortical slices incubated under identical conditions. Accordingly, in the present in vitro studies, we examined the effects of O_2 on basal and hormone—responsive cAMP in medullary and cortical slices from rat kidney. Evidence was obtained that: (*a*) the cAMP content of inner medulla increases progressively as a function of the O_2 content of the gas phase; and (*b*) this effect of O_2 may be mediated at least in part by local synthesis of prostaglandins.

METHODS

Male Sprague-Dawley rats (Zivic Miller Laboratories Inc., Pittsburgh, Pa.), weighing 300–350 g, were fasted 18 h and anesthetized with pentobarbital (5 mg/100 g body weight i.p.). The kidneys were excised and placed immediately in 0.85% saline at 0–4°C. The renal capsules were removed manually and slices of cortex and medulla prepared with a Stadie-Riggs microtome. Cortex, outer and inner medulla were further separated by careful dissection of each slice on a filter paper moistened with cold saline. Slices were incubated at 37°C in 2 ml of Krebs Ringer bicarbonate buffer (pH 7.4) containing 1 mg/ml of glucose and bovine serum albumin (KRBG) in the presence or absence of 2 mM 1-methyl-3-isobutylxanthine (MIX) and (or) other test agents, as indicated in the Results. Incubations were conducted in 25-ml flasks in a Dubnoff metabolic incubator agitated at 100 cycle/min. The gas phase consisted of 5% CO_2 plus the variable proportions of O_2 and N_2 indicated in the Results. It should be emphasized that efficient agitation of inner medullary slices was essential to demonstrate the O_2-dependent changes in cAMP reported in this study. At the conclusion of timed incubations, slices were either extracted for cAMP or ATP content or homogenized in appropriate buffers for determination of adenylate cyclase or cAMP-phosphodiesterase activities, as outlined below.

Extraction and assay of tissue cAMP and ATP. cAMP was extracted by homogenizing individual slices in 0.5 ml of 50 mM sodium acetate buffer (pH 4.0) at 95°C and heating the homogenate for an additional 10 min at this temperature. Homogenates were spun at 3,300 *g* for 10 min at 4°C in an International Centrifuge (International Equipment Co., Boston, Mass.) and appropriate dilutions of the resultant supernatant fractions assayed directly for cAMP by the protein binding method, as previously described (11). Approximately 40–70 mg of cortex, 30–50 mg of outer medulla, or 15–25 mg of inner medulla were extracted. cAMP content of each slice extract was determined in duplicate samples. These duplicate determinations from each slice were then averaged and entered as a single value for purposes of statistical analysis. Authenticity of the cAMP determinations in all three tissues was verified by the linearity of sample dilutions, complete destruction of assayable cAMP by prior treatment of tissue extracts with excess phosphodiesterase, and by the quantitative recovery of exogenous "cold" cAMP added to tissue homogenates. cAMP recovery was monitored by addition of 2,000 cpm of [^{3}H]cAMP to the extraction buffer.

ATP content was extracted by rapidly homogenizing slices in 5% perchloric acid at 0–4°C. Values for ATP obtained in slices processed in this manner were not different from those observed when slices were quick-frozen in liquid N_2 and homogenized as a frozen powder. Supernatant fractions of these homogenates, prepared as described above, were neutralized by addition of KOH. ATP was determined by the luciferin-luciferase bioluminescent method as previously reported (11, 12). A single slice of approximately 50 mg was extracted for each assay of ATP, with duplicate samples of each extract then assayed.

Assay of adenylate cyclase and cAMP-phosphodiesterase activities. For assay of adenylate cyclase activity approximately 100 mg of tissue was homogenized in 1 ml of 10 mM Tris buffer (pH 7.4, 0–4°C) containing 0.2 mM EDTA. Enzyme activity was determined as previously reported (11). Briefly, cyclase reaction mixtures contained 2 mM ATP, 2×10^6 cpm [α-^{32}P]ATP, 1 mM cAMP, 5 mM $MgSO_4$, 50 mM Tris (pH 7.6), 20 mM caffeine, 25 mM creatine phosphate, 0.5 mg/ml of creatine phosphokinase, and 1.3 mg/ml bovine serum albumin. Reactions were initiated by addition of 200–300 μg protein from whole homogenates of cortex or inner medulla and incubated for 7 min at 30°C. cAMP formation was linear with respect to time for at least 10 min and with respect to added protein (100–400 μg) under these assay conditions. cAMP was isolated by the two step column chromatographic procedure (AG 50W-X4 followed by alumina), as previously described (11).

For assay of cAMP-phosphodiesterase activity approximately 75 mg of tissue was homogenized in 50 mM Tris buffer (pH 7.4, 0–4°C) containing 0.25 M sucrose. Enzyme activities of whole homogenates of the slices were assayed at 30°C as previously outlined (11), with the general method of Thompson and Appleman (13) with one modification. [^{14}C]adenosine (3,000 cpm) was added just before the treatment of samples with snake venom, to assess loss of adenosine through absorption to AG 1-X2 resin, as recommended by Boudreau and Drummond (14). This loss was consistently 35–40% of the added [^{14}C]adenosine, and final results were corrected accordingly. cAMP hydrolysis was determined at two substrate concentrations (0.1 μM and 0.1 mM) with sufficient tissue added to destroy 20% of substrate. Hydrolysis was linear for the 5-min assay period, with results expressed as pmol cAMP hydrolyzed/5 min per mg protein. Protein was determined by the method of Lowry et al. (15).

Statistical significance of differences between mean values was determined with Student's *t* test for unpaired values. In all instances *n* values refer to the number of individual slices studied, whether from a single experiment or pooled from several replicate experiments. When results from a single representative experiment are presented, the experiment was repeated at least twice. All differences between mean values with *P* at least <0.05 are indicated in the tables and figures.

Materials. Arginine vasopressin (AVP) (sp act 367 U/mg, Grade VI, synthetic), parathyroid hormone (PTH) (sp act 210 U/mg), and indomethacin were obtained from Sigma Chemical Co., St. Louis, Mo. prostaglandin E_1 (PGE_1) was a generous gift of Dr. John Pike, The Upjohn Co., Kalamazoo, Mich. Sodium meclofenamate was kindly donated by Dr. James E. Gleichert, Parke, Davis and Co., Ann Arbor, Mich. MIX was purchased from Aldrich Chemical Co., Milwaukee, Wis. [^{14}C]adenosine (sp act, 40–60 mCi/mmol), [α-^{32}P]ATP (sp act 20 Ci/mmol), and [^{3}H]cAMP (sp act), 38.4 Ci/mmol) were purchased from New England Nuclear, Boston, Mass. Sources of all other materials have been previously reported (11, 12).

RESULTS

Table I compares the cAMP and ATP content of slices of rat renal cortex, outer and inner medulla, and

TABLE I

cAMP and ATP Content of Slices of Rat Liver, Renal Cortex, and Medulla Exposed to High Oxygen Tension

Tissue	cAMP	ATP
	pmol/mg wet weight	*μmol/g wet weight*
Liver	0.36±0.03	0.99±0.10
Renal cortex	0.43±0.03	1.13±0.12
Renal medulla		
Outer	2.04±0.22*	1.06±0.09
Inner	4.45±0.31*‡	0.94±0.10

Tissue slices were incubated for 20 min at 37°C in KRBG (no MIX) with 95% O_2 and 5% CO_2 serving as the gas phase. At the conclusion of the incubation, tissue cAMP and ATP content was extracted by homogenizing individual slices in 50 mM sodium acetate buffer (pH 4.0) at 95°C or 5% perchloric acid at 4°C, respectively. Each value represents the mean ±SE determinations on eight slices of each tissue from a single representative experiment.

* $P < 0.001$ vs. values in liver or renal cortex.

‡ $P < 0.001$ comparing values in inner and outer medulla.

liver after incubation of these tissues for 20 min in KRBG with a gas phase consisting of 95% O_2 + 5% CO_2. cAMP levels of both outer and inner medulla were significantly greater than those of cortex or liver, whereas ATP content of all four tissues was comparable at the conclusion of the incubation. The relative differences in cAMP observed under these conditions at 20 min persisted for periods of at least 2 h (not shown). cAMP levels were consistently highest in the inner medulla (6–10-fold above cortical values). Accordingly, cAMP metabolism was subsequently examined in greater detail in inner medulla and compared to that of cortex.

TABLE II

Effects of Oxygen on the cAMP Content of Renal Cortex and Inner Medulla

Test agent	95% O_2 + 5% CO_2		95% N_2 + 5% CO_2	
	Cortex	Inner medulla	Cortex	Inner medulla
	pmol/mg wet weight			
None	0.51±0.03	5.18±0.46	0.36±0.04*	1.62±0.23*
MIX	2.57±0.24‡	22.5±2.48‡	1.41±0.22‡*	4.37±0.41‡*
MIX + PGE_1	4.50±0.38§	29.1±2.72§	1.67±0.25*	7.14±0.68§*
MIX + AVP	—	59.4±5.28§	—	24.5±2.10§*
MIX + PTH	87.3±8.4§	—	1.93±0.28*	—

Slices of renal cortex and inner medulla were incubated for 20 min at 37°C in KRBG with either 95% O_2 + 5% CO_2 or 95% N_2 + 5% CO_2 serving as the gas phase. MIX (2 mM) was present in the incubate except where indicated. PGE_1 (0.1 mM), AVP (30 nM), and PTH (2 μM), added at concentrations which gave maximal increases in cAMP, were present for only the final 5-min of the incubation. Each value represents the mean±SE of determinations on six slices from a single representative experiment.

* P at least <0.05 comparing value at 95% N_2 to corresponding value from the same tissue at 95% O_2.

‡ $P < 0.001$ comparing basal values with and without MIX for the same gas phase and tissue.

§ P at least <0.05 comparing value for MIX + PGE_1, AVP or PTH to the value for MIX alone for the same gas phase tissue.

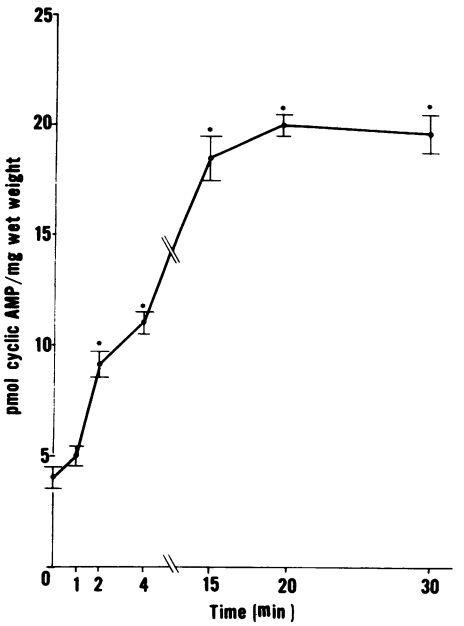

FIGURE 1 Time course of the effects of 95% O_2 on the cAMP content of slices of inner medulla. Slices were initially incubated for 30 min at 37°C in KRBG containing 2 mM MIX, with 95% N_2 + 5% CO_2 serving as the gas phase. At time 0, the latter was replaced with 95% O_2 + 5% CO_2 and the incubations continued for the times shown. Each value represents the mean±SE of determinations on six slices from a single representative experiment. *, P at least <0.02 compared to values without O_2 (time 0).

Table II shows cAMP in cortex and inner medulla after 20-min incubations of these tissues at either high O_2 tension (95% O_2 + 5% CO_2) or without O_2 (95% N_2 + 5% CO_2). At 95% O_2, cAMP in the inner medulla was approximately eightfold higher than in cortex, both with or without 2 mM MIX in the incubation media. The lower tissue cAMP in cortex was not due to a greater egress of the nucleotide into the media. With MIX, cAMP in the media of inner medullary incubates expressed on the basis of tissue wet weight was fourfold higher than that of cortical media at 20 min. Exclusion of O_2 reduced basal cAMP in both tissues, whether levels were examined with or without MIX (Table II). However, in cortical slices deprived of O_2 for 20 min, cAMP without MIX was 0.51±SE 0.03 pmol/mg wet weight at 95% O_2 compared to 0.36±0.04 during O_2 deprivation ($P < 0.05$). By contrast, inner medullary cAMP without MIX was 5.18±0.46 at 95% O_2 and 1.62±0.23 during O_2 deprivation ($P < 0.001$). Effects of O_2 deprivation on the cAMP content of the two tissues were qualitatively similar when MIX was present (Table II). Further, media cAMP also fell 70–80% in inner medullary incubates

when O_2 was excluded (not shown). Despite the greater fall in inner medullary cAMP during O_2 deprivation, absolute levels of cAMP in this tissue remained above those of similarly treated cortex (Table II).

The action of O_2 to increase basal cAMP in inner medulla after a period of O_2 deprivation was rapid. In medullary slices initially incubated without O_2 for 20 min and then exposed to 95% O_2 for timed intervals (Fig. 1), cAMP increased twofold in 2 min and rose to a peak level fivefold over the O_2-deprived basal value in 15–20 min. In contrast to these changes, cAMP increased only 45% in 20 min in identically treated cortex during reoxygenation (1.25±0.19 pmol/mg wet weight in O_2-deprived slices vs. 2.29 ±0.28 after exposure to 95% O_2 for 20 min). Moreover, as shown in Fig. 2, inner medullary cAMP rose progressively and significantly during exposure to atmospheric O_2 contents ranging from 0 to 20%. Although inner medullary oxygen tension was not determined directly in the present study, the atmospheric O_2 contents studied (0, 5, 10, and 20%) should produce and encompass the range of O_2 tensions likely to pertain in this tissue in vivo (7–9).

O_2 deprivation also differentially altered the responses of cortex and inner medulla to agonists of cAMP. Incubation of cortical slices without O_2 for 20 min completely abolished the increases in cAMP mediated by concentrations of PTH (2 μM) and PGE_1 (0.1 mM) that gave maximal stimulation in the presence of O_2 (Table II). In the inner medulla, O_2-deprivation markedly reduced the absolute accumulation of cAMP in response to a maximally effective concentration of either AVP or PGE_1. However,

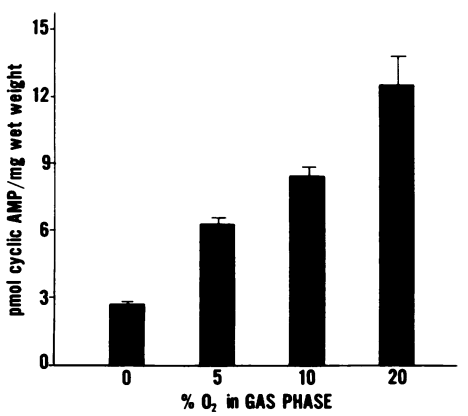

FIGURE 2 Effects of varying atmospheric O_2 content from 0 to 20% on cAMP in inner medullary slices. Slices were incubated for 20 min at 37°C in KRBG containing 2 mM MIX. The O_2 content of the gas phase was as indicated. Each value represents the mean±SE of determinations on 24 slices (quadruplicate slices pooled from six separate experiments). With each increment in O_2, cAMP rose significantly (P at least <0.02 compared to the proximate value).

TABLE III

Effect of Indomethacin on the cAMP Content of Renal Cortex and Inner Medulla Exposed to High Oxygen Tension

	Cortex		Inner medulla	
Test agent	Indomethacin		Indomethacin	
	pmol cAMP/mg wet weight			
	−	+	−	+
None	0.47±0.04	0.56±0.05	4.35±0.51	2.86±0.31*
MIX	2.63±0.22	2.79±0.23	30.9±3.21	9.34±1.12*
MIX + PGE_1	4.81±0.53‡	5.68±0.51‡	28.4±3.02	25.9±2.13‡
MIX + PTH	75.3±7.92‡	92.6±8.45‡	—	—
MIX + AVP	—	—	69.7±5.44‡	65.6±5.17‡

Slices of renal cortex and inner medulla were incubated for 20 min at 37°C in KRBG with 95% O_2 + 5% CO_2 serving as the gas phase. MIX (2 mM) and indomethacin (100 μg/ml) were present as indicated. PGE_1 (0.1 mM), AVP (30 nM), and PTH (2 μM) were present for only the final 5-min of the incubation. Each value represents the mean±SE of determinations on six slices from a single representative experiment.

* P at least <0.01 compared to value from the same tissue incubated in the absence of indomethacin.

‡ P at least <0.01 comparing value for PGE_1, AVP, or PTH to value for MIX alone for the same tissue and incubation condition.

medullary responses to these agonists were not abolished by O_2 deprivation. Indeed, relative increases in cAMP over the basal in response to either maximal PGE_1 or AVP were enhanced approximately twofold during O_2 exclusion. Similarly, a submaximal concentration of AVP (3 nM) increased cAMP approximately 1.7-fold over the corresponding basal at 95% O_2 and approximately 2.8-fold over the O_2 deprived basal. However, in both instances, the apparent enhancement of AVP responsiveness during O_2 exclusion was a function of the marked decline in basal cAMP under these conditions. Absolute cAMP accumulation with submaximal or maximal AVP stimulation was greatest at 95% O_2. Addition of exogenous PGE_1 to O_2-deprived inner medullary incubates containing MIX failed to increase cAMP to the absolute basal levels observed at 95% O_2 in the presence of MIX (Table II). This was also the case when effects of exogenous PGE_1 on cAMP were examined during O_2 exclusion in the absence of MIX (not shown).

cAMP content of inner medullary slices exposed to a high atmospheric O_2 resembled hormonally stimulated values seen in renal cortex and other tissues. Relative to cortex, the inner medulla is known to be an active site of prostaglandin synthesis (16–19) and this process utilizes O_2 as substrate (20). Furthermore, prostaglandins are recognized agonists of the adenylate cyclase-cAMP system in several tissues, including renal medulla (21). In view of these considerations it seemed possible that locally generated prostaglandins might be involved in the action of O_2 on inner medullary cAMP. To examine this question, inner medulla was incubated for 20 min at 95% O_2 with and without 100 μg/ml indomethacin. In incubations conducted in both the presence and absence of

TABLE IV

Effects of Sodium Meclofenamate on the cAMP Content of Inner Medullary Slices Incubated at High Oxygen Tension

Test agent	Meclofenamate	
	pmol cAMP/mg wet weight	
	−	+
None	36.8±4.9	10.7±2.4*
AVP	75.8±8.2‡	82.3±8.6‡
PGE_1	40.7±5.1	37.5±4.4‡

All slices were incubated for 20 min at 37°C in KRBG buffer (gas phase 95% O_2 + 5% CO_2) containing 2 mM MIX in either the presence or absence of sodium meclofenamate (50 μg/ml) as indicated. AVP (30 nM) and PGE_1 (0.1 mM) were present for the final 5-min of the incubation. Values represent means±SE of determinations on six slices from a single representative experiment.

* $P < 0.001$ vs. corresponding value without meclofenamate.

‡ $P < 0.001$ vs. value without test agent.

MIX, indomethacin significantly suppressed the basal cAMP content of inner medulla (Table III). cAMP levels with indomethacin did not fall to those observed during 20 min of O_2 exclusion (Table II). The action of indomethacin on cAMP was not related to depletion of tissue ATP (control ATP, 0.92±SE 0.11 μmol/g wet; indomethacin, 0.96±0.10; $n = 5$). Reductions in basal medullary cAMP were maximal in response to 100 μg/ml of indomethacin. This concentration of the drug did not alter absolute accumulation of cAMP by medullary slices incubated with AVP or PGE_1, or influence basal, PTH, or PGE_1-responsive cAMP in cortical slices (Table III). Indeed, addition of a maximally effective concentration of exogenous PGE_1 to incubates containing indomethacin increased absolute cAMP content of inner medulla to a value (25.9±SE 2.13 pmol/mg wet weight) which approximated that seen at 95% O_2 without the blocker (30.9±3.21). Moreover, cAMP responsiveness of inner medulla to PGE_1 was much more apparent in the presence of indomethacin (Table III). In separate studies conducted at 95% O_2 without MIX, PGE_1 similarly restored absolute cAMP levels of indomethacin-treated inner medulla (95% O_2, 5.12±SE 0.60 pmol cAMP/mg wet weight; 95% O_2 + indomethacin, 3.49±0.37; 95% O_2 + indomethacin + PGE_1, 5.34±0.55, with n of six slices for each group). Sodium meclofenamate (50 μg/ml), an inhibitor of prostaglandin synthesis which is structurally distinct from indomethacin (22), had effects on basal, AVP and PGE_1-responsive cAMP of inner medulla that were qualitatively identical to the latter drug (Table IV). This concentration of meclofenamate also did not alter ATP levels in inner medulla, or basal and hormone-stimulated cAMP in cortex (not shown). Although higher concentrations of meclofenamate further reduced basal cAMP in inner medulla, this may have been due to tissue injury, since ATP also fell and absolute cAMP responses to AVP were blunted. As was the case with indomethacin, meclofenamate failed to lower inner medullary cAMP levels to those observed during O_2 deprivation (Table II). However, after addition of exogenous PGE_1 (Table IV), cAMP content of slices incubated at 95% O_2 with meclofenamate (37.5±SE 4.4 pmol/mg wet weight) did not differ from that of slices incubated at 95% O_2 without test agents (40.7±5.1). cAMP responsiveness of the inner medulla to PGE_1 was also readily apparent in the presence but not the absence of meclofenamate (Table IV).

Fig. 3 shows the effects of indomethacin on inner medullary cAMP as a function of the O_2 content of the gas phase during 20-min incubations. As the O_2 content of the gas phase was increased from 0 to 50%, cAMP rose progressively from 4.13±0.38 to 18.2±1.8 pmol/mg wet weight. The correlation coefficient between cAMP and O_2 from 0 to 50% was 0.92, suggesting a linear relationship ($P < 0.005$). In the absence of O_2, indomethacin did not detectably

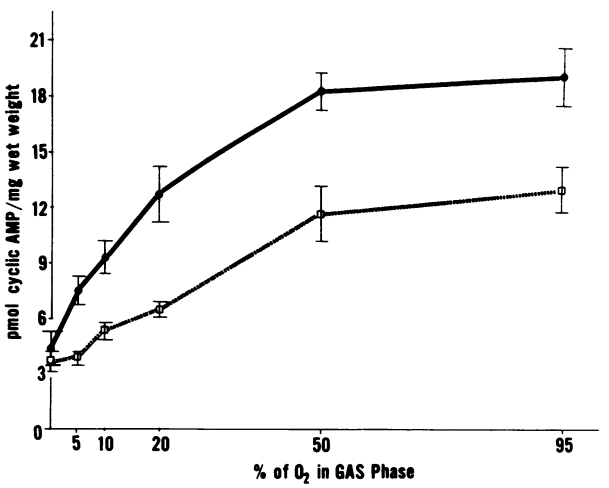

FIGURE 3 Effects of O_2 on the cAMP content of renal inner medulla in the presence (□ . . . □) and absence (● —— ●) of indomethacin. Slices were incubated for 20 min at 37°C in KRBG containing 2 mM MIX, with or without indomethacin (100 μg/ml). The O_2 content of the gas phase was varied as indicated. The residual gas phase consisted of N_2 + 5% CO_2. Each value represents the mean±SE of determinations on five slices from a single representative experiment. In the absence of indomethacin, all cAMP values in oxygenated slices were significantly higher than the value in O_2 deprived slices (P at least <0.05). Indomethacin detectably (P at least <0.02) reduced cAMP content in all oxygenated slices compared to the value observed at the corresponding O_2 content without indomethacin, while in O_2-deprived slices indomethacin did not significantly alter cAMP.

influence inner medullary cAMP. However, at all O_2 concentrations tested from 5 to 95%, cAMP of inner medulla was significantly lower in the presence than in the absence of indomethacin. Consequently, the slope of the O_2-cAMP response curve (0.29 pmol/mg wet weight per % O_2) was clearly reduced by indomethacin (0.16), $P < 0.01$.

Table V summarizes changes in adenylate cyclase activities in whole homogenates prepared from cortical and inner medullary slices which had been previously incubated for 20 min in KRBG in the presence or absence of 50 μg/ml of meclofenamate. Slices were homogenized at 4°C and enzyme activity assayed immediately. The basal, maximal NaF-, and PTH-responsive adenylate cyclase activities of cortical slices preincubated with meclofenamate did not differ from the corresponding values in slices without prior exposure to this agent. By contrast, preincubation of inner medullary slices with meclofenamate significantly reduced basal adenylate cyclase activity, but did not alter absolute NaF-, AVP- or PGE_1-responsive activities. However, even in tissue with prior exposure to meclofenamate, specific activity of adenylate cyclase in inner medulla remained significantly above that of cortex. This was also the case in O_2 deprived inner medulla (not shown). As is shown in Table V, addition of a maximally effective concentration of PGE_1 to reaction mixtures containing tissue previously incubated with meclofenamate at 95% O_2 increased enzyme activity to levels (102±SE 11 pmol cAMP formed/min per mg protein) comparable to those observed in homogenates of inner medullary slices incubated at 95% O_2 without the blocker (83±7). PGE_1-responsive adenylate cyclase activity was clearly evident in inner medulla which had been previously incubated with meclofenamate, but not in tissue without exposure to the drug (Table V). In a separate experiment, slices of inner medulla were initially incubated with 50 μg/ml of meclofenamate for 20 min and then in the presence or absence of 0.1 mM PGE_1 for a final 5 min. Basal adenylate cyclase activity determined in rapidly prepared homogenates was 54±5 pmol cAMP/min per mg protein in slices without, and 86±9 in slices with prior exposure to PGE_1 (mean ±SE of six slices, $P < 0.01$). The latter activity was only 100±11 in the presence of 0.1 mM fresh PGE_1 in the reaction mixture, an insignificant increase statistically. These results imply residual PGE_1 or PGE_1-effect in the homogenates prepared from slices with prior exposure to the agonist, a phenomenon which has been observed previously in hepatic slices preincubated with high concentrations of glucagon (11). Such carry-over of prostaglandin effect may account for the elevated basal adenylate cyclase activity of homogenates prepared from inner medullary slices incubated at 95% O_2 in the absence of meclofenamate (Table V).

TABLE V

Effects of Prior Incubation of Renal Cortical and Inner Medullary Slices with Sodium Meclofenamate on Adenylate Cyclase Activities

Test agent in the cyclase reaction mixture	Cortex Meclofenamate		Inner medulla Meclofenamate	
	pmol cAMP formed/min/mg protein			
	−	+	−	+
None	19±2	17±2	83±7	46±5*
NaF (15 mM)	147±16‡	153±16‡	214±15‡	225±13‡
PTH (2 μM)	99±5‡	92±6‡	—	—
AVP (30 nM)	—	—	193±12‡	187±17‡
PGE_1 (0.1 mM)	—	—	94±9	102±11‡

Slices of cortex and inner medulla were initially incubated at 37°C for 20 min in KRBG in the presence or absence of sodium meclofenamate (50 μg/ml), as indicated. The gas phase was 95% O_2 + 5% CO_2. At the conclusion of this incubation slices were homogenized at 4°C in 10 mM Tris buffer (pH 7.4) containing 0.2 mM EDTA (100 mg tissue/ml buffer). These homogenates were then assayed immediately for adenylate cyclase activity in the presence of the test agents indicated. Concentrations of test agents shown gave maximal increases in enzyme activity. Each value represents the mean±SE of determinations of enzyme activity on four slices from a single representative experiment.

* $P < 0.001$ vs. corresponding value without meclofenamate.

‡ $P < 0.001$ vs. value with no test agent for the same tissue and slice incubation condition.

High (0.1 mM) and low (0.1 μM) Km cAMP-phosphodiesterase activities of homogenates of inner medullary or cortical slices preincubated with meclofenamate did not differ detectably from corresponding activities in slices not exposed to this agent (control low Km activity in cortex, 154±17 pmol cAMP hydrolyzed/5 min per mg protein; inner medulla, 143±15). Direct addition of meclofenamate or indomethacin (100 μg/ml) to phosphodiesterase reaction mixtures significantly inhibited cAMP degradation by homogenates of both tissues (not shown).

DISCUSSION

The results of the present studies demonstrate that the cAMP content of inner medulla is rapidly and dramatically altered by O_2. These effects of O_2 in inner medulla were quite distinct from those observed in cortex, although qualitatively similar changes were observed in outer medulla. When O_2-deprived inner medulla was exposed to 95% O_2, cAMP levels rose 4 to 6-fold in 20 min, reaching values 6 to 10-fold higher than those observed in cortex at 95% O_2. While cAMP accumulation in inner medulla increased progressively as the O_2 content of the gas phase of incubation vessels was increased from 0 to 50%, only modest differences were noted in the

basal cAMP levels of renal cortical slices incubated with or without O_2 for 20 min. The action of O_2 to increase cAMP in inner medulla was clearly evident in the presence of MIX, a potent inhibitor of phosphodiesterase activity, and was associated with higher levels of cAMP in the incubation media. Furthermore, basal adenylate cyclase activity of inner medullary slices incubated at 95% O_2 was significantly higher than that of cortex, and activity of only the former was appreciably inhibited by preincubation of slices with meclofenamate. By contrast, differences in the basal phosphodiesterase activities of inner medulla and cortex were not evident at 95% O_2. These observations all indicate that the enhanced cAMP accumulation in inner medulla at high O_2 is due to an acceleration of nucleotide generation from the elevated adenylate cyclase activity, rather than to a reduced rate of cAMP hydrolysis or decreased cAMP efflux from the tissue to media. Previous comparisons of the cAMP levels of renal cortex, outer and inner medulla have failed to reveal the marked differences in basal cAMP content observed in the present studies of 95% O_2, although a relatively high cAMP content has been noted in the inner medulla (23, 24). Since the cAMP content of the latter is greatly altered by O_2, the lower cAMP levels reported in earlier studies of this tissue could reflect differences in tissue oxygenation. In the present experiments, specific precautions were taken to insure ample O_2 availability during in vitro incubations by agitating small tissue slices in 25-ml flasks containing only 2 ml of buffer and 23 ml of 95% O_2. Under such conditions, progressive enhancement of inner medullary cAMP content was demonstrated in response to changes in atmospheric O_2 content from 0 to 20%. This range of atmospheric O_2 should result in and encompass O_2 tensions which might pertain in vivo in inner medulla. The latter has been estimated to be 30–50 mm Hg (7–9).

There is considerable evidence that the inner medulla is an active site of prostaglandin synthesis (16–19) and the present data imply that the action of O_2 to augment cAMP in this tissue may be related at least in part to local generation of prostaglandin. Indomethacin, an inhibitor of prostaglandin synthesis, markedly suppressed the effects of O_2 to increase cAMP in inner medulla in response to a wide range of O_2 tensions. Meclofenamate, another inhibitor of prostaglandin synthesis which is structurally different from indomethacin (22), also significantly reduced the cAMP content of inner medulla at high O_2 tension and concomitantly lowered basal adenylate cyclase activity of this tissue. The actions of indomethacin and meclofenamate to suppress the basal activity of the adenylate cyclase-cAMP system of inner medulla were not toxic effects, since (*a*) these actions were specific for inner medulla relative to cortex and (*b*) absolute increases in adenylate cyclase activity or cAMP in response to agonists were not altered in either tissue. Indomethacin has been reported to inhibit phosphodiesterase activity in some tissues (25) and to suppress oxidative phosphorylation in hepatic mitochondria (26). However, such actions could not be implicated in the ability of either indomethacin or meclofenamate to lower inner medullary cAMP. Thus, the ATP levels of inner medulla were not influenced by indomethacin or meclofenamate at concentrations which clearly suppressed cAMP, while inhibition of cAMP hydrolysis, which could be demonstrated upon direct addition of indomethacin or meclofenamate to phosphodiesterase reaction mixtures, would result in an increase rather than a decline in tissue cAMP. Moreover, the addition of maximally effective concentrations of PGE_1 to inner medullary slices which had been previously incubated with indomethacin or meclofenamate at 95% O_2 restored cAMP to levels which approximated those observed in tissue exposed to 95% O_2 alone (Tables III and IV). Similarly, a maximally effective concentration of PGE_1 increased the adenylate cyclase activity of inner medullary homogenates with prior exposure to meclofenamate and 95% O_2 to levels comparable to those observed in tissue previously exposed to O_2 alone (Table V). Responsiveness of the adenylate cyclase-cAMP system of inner medulla to exogenous PGE_1 was readily apparent in the presence, but not in the absence, of blockers of prostaglandin synthesis, presumably reflecting a decline in the endogenous prostaglandin content of this tissue mediated by the drugs. O_2 deprivation also led to a relative enhancement of cAMP responses to both exogenous PGE_1 and vasopressin (Table II). However, this phenomenon was difficult to interpret due to the marked decline in absolute cAMP which occurred when O_2 was excluded.

Thus, the present observations provide substantial indirect evidence that local synthesis of prostaglandin is involved in O_2-mediated increases in inner medullary cAMP. Recent preliminary studies by Levitt[2] have provided additional direct support for this hypothesis. In these experiments, inner medullary slices were incubated for 20 min at 37°C at either 95% or 5% O_2 under conditions otherwise identical to those described in the present report, and the accumulation of immunoreactive PGE in the media determined by techniques previously reported (27). Media PGE content was threefold higher at 95% O_2 (33.5±4.8 ng/mg inner medulla) than at 5%

[2] Personal communication, Dr. M. J. Levitt, Department of Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, Pa.

(11±0.9 ng/mg), $P < 0.001$ with six slices in each group.[2] The mechanism by which O_2 enhances generation of prostaglandin in inner medulla is currently undefined. This effect may occur in other tissues (28). In the biosynthesis of prostaglandins, O_2 is utilized as substrate by prostaglandin synthetase for the oxidation of arachidonic or linoleic acids (20). Thus, it is possible that O_2 availability is rate limiting in this biosynthetic step. If this is the case, then factors which alter the ordinarily low O_2 tensions of inner medulla in vivo, such as changes in vasa recta blood flow (7–9), might also influence local prostaglandin synthesis and cAMP-mediated medullary function. Changes in cAMP in vitro occurred over a range of atmospheric O_2 from 0 to 20% and, thus, in vivo physiologic correlates of this action seem possible. Although the existence of such correlates are not established by our data, nor reported in the literature, in vivo examination of cAMP, prostaglandins and cAMP-mediated inner medullary functions in response to altered O_2 tensions would be of considerable interest.

Finally, although the effects of O_2 on inner medullary cAMP are likely mediated in part by local prostaglandins, these O_2 actions were complex and additional factors may be involved. Thus, cAMP generation in inner medulla was severely limited during O_2 exclusion, and exposure of O_2-deprived medullary slices to exogenous PGE_1 failed to restore cAMP to levels observed in oxygenated slices, as might be expected if O_2-dependent prostaglandin synthesis were the exclusive determinant of high basal cAMP in this tissue. Moreover, blockers of prostaglandin synthesis did not suppress the cAMP content of oxygenated inner medullary slices to levels observed in O_2-deprived slices of this tissue, further evidence against prostaglandins as sole factors in the maintenance of high steady-state cAMP levels in oxygenated inner medulla. In part, the differences in cAMP content between inner medulla and cortex observed both with and without O_2 may be a direct function of the high specific activity of adenylate cyclase in inner medulla and represent a property of this tissue which is independent of effects of either O_2 or prostaglandins. Whatever the ultimate mechanisms involved in expression of the effects of O_2 on inner medullary cAMP, it is clear that the conditions of oxygenation must be carefully considered in future studies of cAMP metabolism in this tissue.

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