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Action of serotonin (5-hydroxytryptamine) on cyclic nucleotides in glomeruli of rat renal cortex

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Action of serotonin (5-hydroxytryptamine) on cyclic nucleotides in glomeruli of rat renal cortex. Serotonin (5-hydroxytryptamine) is known to influence glomerular function and may have an important role in the pathogenesis of glomerulopathies. Because serotonin acts in nonrenal tissues through mediation of cyclic nucleotides, we investigated in vitro its effect on cAMP and cyclic guanosine monophosphate (cGMP) in tissue slices and isolated glomeruli from rat kidney. Serotonin increased cAMP 161 ± 35% but not cGMP in renal cortex; it had no effect on cyclic nucleotides in medulla and papilla. In isolated glomeruli, serotonin elicited a dose-dependent (in the range of 10^{-7} to 10^{-4} M) increase in cAMP; the maximum increase over basal values was 376 ± 45%. Serotonin increased cAMP either in the presence or in the absence of a cAMP phosphodiesterase inhibitor. In tubular fraction, serotonin elevated cAMP to a much lesser degree (82 ± 15%). Neither in glomeruli nor in tubules did cGMP concentrations change in response to serotonin, but carbamylcholine, a known cGMP agonist, significantly increased cGMP concentrations. The increase in cAMP in response to serotonin was blocked (>85% inhibition) by equimolar concentrations of serotonin antagonists methysergide and cinanserine. Results of this study demonstrate that interaction of serotonin with receptors in the kidney, particularly in the glomeruli, cause a striking increase in cAMP concentrations without detectable changes in cGMP concentrations. These findings suggest that serotonin, either synthesized in the kidney or released locally from platelets aggregated in glomeruli (for example, in association with immunopathologic injury) may exert or modulate its physiologic or pathologic effects via mediation of cAMP.

Action de la sérotonine (5-hydroxytryptamine) sur les nucléotides cycliques des glomérules du cortex rénal du rat. Il est connu que la sérotonine (5-hydroxytryptamine) influence la fonction glomérulaire et peut avoir un rôle important dans la pathogénie des glomérulopathies. Puisque la sérotonine agit sur d'autres tissus que le rein par l'intermédiaire des nucléotides cycliques, nous avons étudié son effet in vitro sur le contenu en cAMP et cGMP des tranches de rein et des glomérules isolés du rein de rat. La sérotonine augmente cAMP 161 ± 35%, mais pas cGMP du cortex rénal. Elle n'a pas d'effet sur les nucléotides cycliques de la médullaire et de la papille. Dans les glomérules isolés la sérontonine détermine une augmentation de cAMP dose dépendante (de 10^{-7} à 10^{-4} M), l'augmentation maximale est de 376 ± 45% par rapport à la valeur basale. La sérotonine augmente cAMP aussi bien en présence qu'en l'absence d'inhibiteur de la cAMP phosphodiestérase. Dans les fractions tubulaires la sérotonine n'augmente cAMP qu'à un moindre degré (82 ± 15%). La sérotonine n'augmente cGMP ni dans les glomérules ni dans les tubules mais la carbamylcholine, un agoniste connu de cGMP augmente celui-ci significativement. L'augmentation de cAMP en réponse à la sérotonine est bloquée (inhibition supérieure à 85%) par des concentrations équimolaires des antagonistes méthysergide et cinansérine. Les résultats de ce travail démontrent que l'interaction de la sérotonine avec les récepteurs rénaux, particulièrement glomérulaires, détermine une augmentation importante de cAMP sans modification détéctable de cGMP. Ces constatations suggèrent que la sérotonine, qu'elle soit synthétisée dans le rein ou libérée localement par des aggrégats plaquettaires formés dans les glomérules (c'est à dire en association avec une lésion immunopathologique), peut exercer ou moduler ses effets physiologiques ou pathologiques par l'intermédiaire de cAMP.

The kidney is one of the tissues with the highest capacity for synthesis and degradation of serotonin (5-hydroxytryptamine) [1], and the kidney has a high content of both serotonin [2] and the serotonin precursor 5-hydroxytryptophan [2]. Serotonin was shown to have a profound effect on different renal functional parameters [1]. Administered through different routes or infused directly into the renal artery, serotonin caused renal vasoconstriction, mainly in the cortex, decreased GFR [1, 3], and alteration of renal handling of fluid and electrolytes, mainly decreases in urine flow and sodium excretion [1]. Serotonin was also reported to stimulate erythropoietin production in the kidney [4].

Perhaps much more important, both experimental and clinical observations suggest that serotonin may be a significant factor in the pathogenesis of major renal disorders, such as glomerulonephritis or other nephropathies [1]. There is increasing evidence that platelets, a major source (>95%) of circulating blood serotonin [5], participate in the development of local glomerular injury occurring in immune complex glomerulonephritis [6] and also in

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other glomerulopathies, such as those associated with intravascular coagulation [7]. Serotonin could be released from platelets which are activated locally in glomeruli and contribute to inflammatory mechanisms. That the platelet serotonin concentrations were lower in patients with lupus erythematosis nephritis was noted recently [8]; in several glomerulopathies mediated by circulating immune complexes, high concentrations of plasma serotonin and low concentrations of platelet serotonin were reported [9].

In spite of the potential importance of serotonin in renal pathophysiology, very little is known about its precise site of action in the kidney and about the cellular mechanism by which serotonin elicits its effects in renal tissue.

In some nonrenal tissues, serotonin has been shown to stimulate formation of cyclic nucleotides, either cyclic 3′5′-AMP (cAMP) or cyclic 3′5′-GMP (cGMP), or both, suggesting that these cyclic nucleotides could play a role of "second messengers" in serotonin actions. In mammalian brain tissue [10, 11] or in vascular endothelial cell line [12], and also in some nonmammalian tissues, such as moluscan myocardium [13], insect salivary gland [14], or in liver fluke [15], serotonin increases cAMP concentrations, or it stimulates adenylate cyclase, or it does both. On the other hand, in mammalian umbilical artery [16], uterine [17] or bronchial [18] smooth muscle, or in monocytes [19] and platelets [20], serotonin has been shown to cause an accumulation of cGMP without changes in the cAMP concentrations. Nevertheless, it is not known whether serotonin may act on cyclic nucleotides in kidney tissue.

In view of these considerations, we examined the serotonin effect on concentrations of cAMP and cGMP in tissue slices from rat kidney cortex, medulla, and papilla, as well as in glomeruli and tubules isolated from rat renal cortex. Our results show that, in vitro, serotonin increases cAMP concentrations in renal cortical slices and elicits a striking accumulation of cAMP in isolated glomeruli without changing the cGMP concentration, but it has no detectable effect on cyclic nucleotides in the medulla and papilla.

Methods

Isolation of glomeruli. Tissues samples were obtained from adult male Sprague-Dawley rats. Each rat, weighing 200 to 250 g, was maintained on an ad lib standard diet (Purina Laboratory Rat Chow, Ralston Purina Co., St. Louis, Missouri) and tap water. After inducing anesthesia with pentobarbital (50 mg/kg of body weight), we inserted a polyethylene catheter (PE-100) into the lower abdominal aorta immediately above the bifurcation. Subsequently, the aorta was clamped above the renal arteries, the renal veins were cut, and the kidneys perfused with 60 to 80 ml of modified Krebs-Ringer phosphate buffer (KRB) of the following composition: 140 mM sodium chloride, 5 mM potassium chloride, 1.2 mM magnesium sulfate, 2.0 mM calcium chloride, 10 mM glucose, 10 mM sodium acetate, 2 mM sodium phosphate, and 20 mM Tris (pH, 7.4). The interval between clamping the aorta and onset of the perfusion did not exceed 20 sec. After the kidney surfaces were completely blanched, the kidneys were removed and placed on an ice-cooled dissecting table. All subsequent preparative steps were performed at 4° C.

Cortical tissues from the kidneys of two to three rats were pooled for one experiment. The cortex was dissected from the kidney and used for preparation of glomeruli and tubule samples. In experiments with tissue slices, the kidney was first carefully divided into cortex, medulla, and papilla, and tissue slices (0.5-mm thick) from these three zones were cut with a Stadie-Riggs tissue slicer [21, 22]. These tissue slices were washed several times in ice-cold KRB. Glomeruli and tubule samples were prepared with a combination of sieving and differential centrifugation. The cortical tissue was minced, strained through a stainless steel sieve (220-μm opening), and subsequently passed through a hypodermic needle (23 gauge, Sherwood Med. Industries). The resulting suspension of cortical tissue was washed four times in KRB by repeated centrifugation at $\times$200g for 2 min to eliminate debris of small fragments floating in the supernatant. The resulting pellet was resuspended in about 50 ml of KRB and passed three to five times by $\times 1g$ gravity filtration sequentially through each of the three nylon sieves (NITEX) with pore-opening sizes of first 390 μm, then 250 μm, and finally 213 μm. The particles retained on the 250-μm sieve consisted of tubular fragments and were collected as a tubular fraction. This tubular fraction, referred to later in the text as "tubules," was washed again on the 250-μm nylon screen with about 60 to 80 ml of KRB to reduce glomerular contamination to a negligible level. To obtain the glomerular fraction, referred to further as "glomeruli," after filtering through a 213-μm sieve, we passed the resulting suspension through a screen cloth (NITEX #25, about 60-μm pore opening); this step allowed debris and small fragments to pass

through, with glomeruli retained on the cloth. The retained glomeruli were recovered by inverting the screen over a glass beaker and by washing the glomeruli out with KRB.

Both glomerular and tubular preparations were then pelleted by centrifugation at ×1500*g* for 5 min. Pelleted fractions were gently resuspended in 4 ml of KRB, transferred in preweighed polyallomer tubes, and centrifuged at ×1500*g* for 5 min; the supernatant was removed by aspiration, and the tubes were weighed again to determine approximate wet weight of the preparations. Glomeruli or tubules were suspended in fresh KRB so that approximately 4 to 6 mg (wet weight) of glomeruli and 8 to 12 mg (wet weight) of tubules were contained in 100 μl of KRB suspension.

The purity of each glomerular suspension was evaluated by light microscopy and counting of glomeruli. An aliquot of glomerular suspension (50 to 100 μl) was mixed on the microscopic slide (10:1, volume per volume) with 0.1% toluidine blue solution in isotonic saline to visualize cellular elements and was examined under higher magnification (×430) to evaluate first the structure of isolated glomeruli and then at the lower magnification (×100) to determine the glomerular count of suspension. Isolated glomeruli were without capsules, and the structure was well preserved. The purity of glomeruli was determined by counting at least 200 particles stained with toluidine blue, and only preparations which contained more than 95% of glomeruli were used for subsequent experiments. Tubular suspension was checked for absence of glomeruli. Glomeruli were also examined microscopically after fixing in 10% formol and staining with hematoxylin-eosin. This examination confirmed the intact structure of glomerular preparations. Criteria for purity of glomeruli were the same as those used in our other studies [21, 22].

Glomeruli prepared with this procedure were morphologically indistinguishable from preparations obtained by the Ficoll density centrifugation method, used in our previous experiments [21, 22]. Nevertheless, the degree of purity required for experiments (see above) was obtained more frequently with this sieving method than it was with the density centrifugation method [21], and also the yield of glomeruli was considerably higher. Tubular fraction obtained with this sieving method was composed of shorter tubular fragments than that obtained with the density gradient method [21, 22]. Glomeruli and tubules prepared by the sieving method showed responsiveness (in terms of changes in cyclic nucleotides) to hormonal agents (histamine, parathyroid hormone, carbamylcholine) analogous to glomeruli and tubules prepared by the density gradient method [22].

Incubations and assays. Incubations of tissue preparations and analyses of cyclic nucleotides were conducted with only minor modifications to the method used in our previous study [22]. Freshly prepared glomeruli (4 to 6 mg, wet weight) and tubules (8 to 12 mg wet weight) were resuspended in 100 μl of ice-cold KRB. In other experiments, cortical, medullary, and papillary tissue slices (10 to 20 mg wet weight) also were suspended in 100-μl aliquots of cold KRB. The tissue slices or suspensions were distributed randomly into 10-ml glass homogenizer tubes (size A, Thomas Scientific Products, Philadelphia, Pennsylvania) kept in crushed ice at 0° C, with 300 μl of KRB having been added to these tubes. These suspensions were first preincubated at 37° C for 20 min in a Dubnoff shaking water bath (120 cycles/min). At the end of the 20-min preincubation period, 50 μl of 5 mM 1-methyl-3-isobutyl xanthine (MIX) dissolved in KRB and either 50 μl of KRB (controls) or hormones and/or drugs dissolved in 50 μl of KRB were added to the various suspensions. These additions were made within an interval of 6 sec. The final volume of each suspension then was 500 μl of KRB with 0.5 mM MIX and the final concentration of various test agents as indicated in the Results section. Stock solutions of hormones or drugs were kept at either −20° C or 0° C as appropriate; dilutions and pH adjustments were made immediately prior to incubation. After the various test agents and MIX were added, the tubes were incubated for an additional 2 min at 37° C in a metabolic shaking water bath. The incubation was terminated by adding 500 μl of ice-cold 10% trichloroacetic acid (TCA) to it and homogenizing it immediately with a Teflon pestle (10 strokes). From the TCA homogenate a small aliquot (20 μl in duplicate) was taken for the determination of protein. The remainder of the TCA homogenate was divided into two equal aliguots for determination of cAMP and cGMP by radioimmunoassay (RIA). Tracer amounts of tritiated cAMP or tritiated cGMP were added to the respective aliquots for monitoring recovery. Precipitated proteins were removed by centrifugation. TCA was removed from the supernatant by repeated extraction (×4) with ethyl ether; remnants of ether were evaporated by heating, and samples were adjusted to neutral pH with 0.5 N sodium hydroxide. Aliquots of extracts were used for determinations of cyclic nucleotides by RIA and for

measurements of recovery. In preliminary experiments, cAMP and cGMP were separated prior to RIA on formate DOWEX-1 columns according to the method of Murad, Manganiello, and Vaughan [23]. Results analogous to other tissue systems [18, 24] were obtained with or without DOWEX-1 column separation, even when the extracts of the same tissue sample were measured simultaneously. Therefore, because of the small quantity of material, extracts were not routinely passed through DOWEX-1 columns prior to cAMP and cGMP determinations by RIA.

Cyclic nucleotides were determined by the RIA originally developed by Steiner, Parker, and Kipnis [25] and modified, to increase the sensitivity, by Harper and Brooker [26] and by Frandsen and Krishna [27]. Samples of tissue extracts and standards for determination of cAMP were acetylated [26], and samples for determination of cGMP were succinylated [27] as described in the original methods [26, 27].

Acetylated or succinylated samples were incubated in 50 mM sodium acetate buffer (pH, 6.2) with anti-cAMP or anti-cGMP antibodies and ^{125}I-succinyl cAMP methyl ester or with ^{125}I-succinyl cGMP methyl ester for 16 to 24 hours at 4° C, and then the free and bound antigens were separated by ammonium sulphate precipitation [25]. Radioactivity of the samples was counted in a gamma counter (Searle Analytical Gamma Counter, model 1285). Specificity and sensitivity of antibodies were examined in preliminary experiments and were found to be within the limits claimed by the manufacturer (Schwartz-Mann Labs., Orangeburg, New York). Also, the increase in sensitivity by acetylation or by succinylation was in agreement with that reported by the authors [26, 27]. With acetylation and succinylation of the standards and samples, the lower limit of sensitivity of RIA was 1 fmole for both cAMP and cGMP. In the absence of added cold cAMP or cGMP, approximately 50 to 60% of labeled antigen was bound to antibodies. Because RIA measurements were done on extracts in which cAMP and cGMP were not separated, the cross-reactivity of RIA for cAMP and cGMP was tested. Succinylated cAMP, even at a concentration 100 times higher, did not interfere with measurements of succinylated cGMP in RIA for cGMP; and conversely, acetylated cGMP, even in a concentration 100 times higher than that of acetylated cAMP, did not interfere with the determination of acetylated cAMP by RIA for cAMP. The validity of cAMP and cGMP measurements by RIA was verified also by the linearity of sample dilutions, by cAMP and cGMP hydrolysis with cyclic nucleotide phosphodiesterase, and by quantitative recovery of added unlabeled cyclic nucleotide to the extracts.

Drug agonists and antagonists used in the present system were tested for interference with cAMP and cGMP determination by RIA. None of the tested agents at concentrations higher than the highest concentrations used in the incubation mixtures interfered with determination of cAMP or cGMP by RIA, or with acetylation or succinylation procedures.

Radioactivity of tritiated tracer nucleotides was determined by liquid scintillation counting. The quantity of tritiated cAMP and tritiated cGMP tracers for recovery measurements which contributed to the final concentration of cyclic nucleotides was calculated and subtracted from each sample. After correction for recovery and after substraction of the added radioactive tracer, the contents of cAMP and cGMP in samples were expressed as picomoles per milligram of protein. In preliminary experiments, we established that total protein in glomerular suspension was linearly proportional to the number of glomeruli in aliquots of samples in which glomeruli were counted under the light microscope.

Because glomeruli, tubules, or slices were homogenized together with the incubation medium, the content of cAMP and cGMP represents a sum of the cyclic nucleotides contained within cells and that which leaked into the incubation medium. Accurate separation of glomeruli from the medium would result in a prohibitive time delay between the end of the incubation and the inactivation of the enzymes by homogenization in TCA. Likewise, the small sample size would make determination of wet weight in the final incubation inaccurate. The content of cyclic nucleotide was expressed in picomoles per milligram of protein in the incubation mixture, which was measured in aliquots of the same TCA-homogenate in which cyclic nucleotides were measured.

The protein contents were determined in aliquots of TCA homogenate after neutralization with sodium hydroxide by the method of Lowry et al [28], as used in our previous studies [21, 22]. Determinations of cyclic nucleotides by RIA, protein determination, and measurements of recovery were run in duplicate or triplicate.

Glomeruli, tubules, or slices from the same pool of kidneys were incubated on the same day under exactly the same conditions with or without added

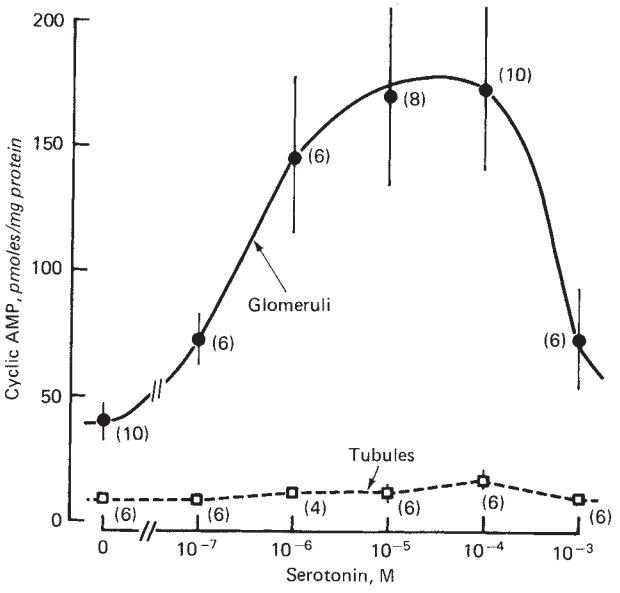

Fig. 1. *Effect of various concentrations of serotonin on cAMP content in glomeruli* (—•—•—) *and in tubules* (—□—□—) *in presence of 0.5 mM MIX.* For experimental details see Methods. Each point represents the mean ± SEM of measurements indicated in parentheses.

hormones or drugs. Samples were processed also (extraction, RIA, and protein determination) at the same time, using the same batch of reagents. This design minimizes possible day-to-day variation between assays and preparative procedures and allows paired comparisons. Glomeruli, tubules, or slices were incubated in duplicate aliquots for each condition (controls or with added drugs or antagonists), and values of duplicates were averaged and entered as a single value (N) for the purpose of statistical analysis. An exception is in the results depicted in Fig. 1 (dose-response curve for serotonin), where each value is based on determination in a single incubation tube.

The effects of drugs and hormones were evaluated as a net difference from control, or as relative (percent change) differences from control, or both, by Student's t test for paired comparisons; other statistical evaluations were done with Student's t test for group comparisons, as indicated in the Results. Values of $P > 0.05$ were considered to be nonsignificant (NS).

Materials. Serotonin (5-hydroxytryptamine creatinine sulphate), histamine, and carbamylcholine and beef heart cyclic nucleotide phosphodiesterase were purchased from Sigma Co., St. Louis, Missouri. Synthetic 1,34-N-terminal tetratriacontapeptide of bovine parathyroid hormone (PTH) activity, 3800 IU/mg) was purchased from Beckman Instruments, Bioproducts, Spinco Division, Palo Alto, California; and 1-methyl-3-isobutyl-xanthine (MIX), from Aldrich Chemical Co., Milwaukee, Wisconsin. Methysergide maleate was a gift from Sandoz Pharmaceuticals, Hanover, New Jersey; cinanserine hydrochloride, a gift from Squibb and Sons, Inc., Princeton, New Jersey; and metiamide, a gift from Dr. Mendelson, Smith, Kline & French, Co., Philadelphia, Pennsylvania. [8-Arginine]-vasopressin (AVP; 385 U/mg) was purchased from Calbiochem, San Diego, California. Nylon meshes for sieving were purchased from Nytex Co., Niles, Illinois, catalog numbers HC3-390 (390-μm pore opening), HC 3-250 (250-μm pore opening), HC 3-212 (212-μm pore opening), and NITEX #25 (pore opening, about 60 μm). Other chemicals, all of the highest purity grades, were purchased from standard suppliers. Tritiated cAMP and tritiated cGMP for measurement of recoveries were purchased from New England Nuclear, Boston, Massachusetts. Antibodies and ^{125}I-labeled antigens were from RIA kits for determinations of cAMP and cGMP purchased from Schwartz-Mann Labs., Orangeburg, New York.

Results

The effect of serotonin on cyclic nucleotides in slices from the three major kidney zones was examined first. The results, summarized in Table 1, show that serotonin increased the cAMP concentration in slices from renal cortex, but under the same conditions, serotonin had no significant effect on cAMP in slices from medulla or papilla of the same kidney. In the same experiments, 10^{-6} M [8-arginine]-vasopressin (AVP) markedly increased cAMP in medullary slices over the basal concentration by 143 ± 28% (mean ± SEM, $N=4$; $P < 0.02$, paired t test), as well as in papillary slices by 205 ± 36% ($N=4$; $P < 0.02$, paired t test). In none of the three kidney zones did serotonin influence tissue concentrations of cGMP. But, 10^{-4} M carbamylcholine, a known stimulant of renal cortical cGMP system [22, 29], elevated the cGMP level by a concentration of 150 ± 35% ($N=6$; $P < 0.01$, paired t test) as anticipated.

In our previous studies, we observed that hormonal agents which cause cyclic nucleotide changes in renal cortical slices can influence differently the concentrations of cAMP or cGMP in glomeruli and tubules [22]. Therefore, the effect of serotonin on cAMP and cGMP in glomeruli (and for comparison in tubules) isolated from renal cortex was examined. As we also noted in our previous studies [22], control (basal) concentrations of both cAMP

Table 1. Effect of serotonin on cyclic nucleotides in slices from renal cortex, medulla, and papilla[a]

	N^b	Control	Serotonin (10^{-4}M)	Relative change from control %
Cortex				
Cyclic AMP, *pmoles/mg protein*	(6)	4.1 ± 0.6	11.0 ± 2.3[c]	+161 ± 35[c]
Cyclic GMP, *pmoles/mg protein*	(6)	0.26 ± 0.04	0.27 ± 0.03	+13 ± 12
Medulla				
Cyclic AMP, *pmoles/mg protein*	(5)	18.6 ± 3.5	20.4 ± 3.1	+17 ± 19
Cyclic GMP, *pmoles/mg protein*	(4)	0.79 ± 0.25	0.77 ± 0.28	−8 ± 13
Papilla				
Cyclic AMP, *pmoles/mg protein*	(5)	117.9 ± 26.3	97.0 ± 27.0	−20 ± 10
Cyclic GMP, *pmoles/mg protein*	(4)	1.92 ± 0.60	1.83 ± 0.77	−10 ± 12

[a] Values represent the mean ± SEM.
[b] N denotes number of experiments.
[c] Values are significantly different from control and/or Δ% is significant at $P < 0.05$ or higher level of significance (paired t test).

Table 2. Effects of serotonin on cyclic nucleotides in glomeruli and tubules[a]

	Glomeruli				Tubules			
	N^b	Control	Serotonin (10^{-4} M)	Relative change from control %	N	Control	Serotonin (10^{-4} M)	Relative change from control
Cyclic AMP, *pmoles/mg protein*	(12)	46.5 ± 9.1	207.1 ± 35.5[c]	+376 ± 45[c, d]	(12)	7.2 ± 0.7	12.6 ± 1.2[c]	+82 ± 15[c]
Cyclic GMP, *pmoles/mg protein*	(12)	4.1 ± 0.9	4.7 ± 0.7	+37 ± 20	(12)	0.44 ± 0.1	0.35 ± 0.06	+18 ± 24

[a] Values represent the mean ± SEM.
[b] N denotes number of experiments.
[c] Values are significantly different from control (control is without addition of serotonin) and/or Δ% change from control is significant at $P < 0.05$ or higher level of significance (paired t test).
[d] The percent increase is significantly ($P < 0.001$; t test) higher than was analogous values in tubules.

and cGMP in glomeruli were, in the present experiments, considerably higher in glomeruli than they were in tubules (Table 2). Serotonin caused a striking increase in cAMP accumulation in glomeruli; statistically significant but much smaller increases both in absolute (net increase) and relative (percent increase) terms were observed in tubules (Table 2). When MIX, a cAMP phosphodiesterase inhibitor, was not included in the incubation medium, the basal concentration of cAMP in incubated glomeruli was much lower (Table 3) than it was with 0.5 mM MIX (Table 2). Nevertheless, even in the absence of MIX, serotonin elicited a marked cAMP increase, and the relative cAMP increase (percent change) was comparable to that observed when 0.5 mM MIX was included (Table 3).

Serotonin had no effect on cGMP either in glomeruli or in tubules. In the same experiments, PTH (20 μg/ml) increased markedly tubular cAMP by 838 ± 264% (mean ± SEM, $N = 5$; $P < 0.025$, paired t test).

In the same preparation of glomeruli in which serotonin did not change cGMP concentrations, 10^{-4} M carbamylcholine increased cGMP by 154 ± 35% (mean ± SEM, $N = 7$, $P < 0.005$, paired t test), and likewise 10^{-4} M histamine caused smaller, but consistent, cGMP elevation by 105 ± 36% (mean ± SEM, $N = 6$, $P < 0.05$, paired t test). Carbamylcholine also increased cGMP in tubules by 110 ± 23% (mean ± SEM, $N = 5$, $P < 0.01$, paired t test) as observed in our previous experiments [22].

As depicted in Fig. 1, the increase in cAMP in glomeruli by serotonin was dose dependent in the range of 10^{-7} to 10^{-4} M. The maximal stimulation occurred in a concentration of 10^{-5} to 10^{-4} M, and a half-maximal increase was elicited by about 3×10^{-7} M serotonin. At the highest concentration of serotonin tested (10^{-3} M), the stimulatory effect decreased. In tubular preparations, a minor cAMP increase occurred at 10^{-4} M serotonin. In the tested range of serotonin concentrations (10^{-7} to 10^{-3} M), no changes in cGMP accumulation either in glomeruli or in tubules were observed. Serotonin antagonists, either methysergide or cinanserine alone in a concentration of 10^{-4} M, did not change cAMP concentrations in glomeruli. Both drugs blocked (more than 85% inhibition) cAMP accumulation in glomeruli (Table 4) elicited by an equimolar concentration

Table 3. Effect of serotonin on cAMP in glomeruli in the absence of cAMP phosphodiesterase inhibitor[a]

	N[b]	Control	Serotonin (10^{-4} M)	Relative change from control %
Cyclic AMP, *pmoles/mg protein*	(5)	5.6 ± 1.9	25.1 ± 8.9[c]	+352 ± 47[c]

[a] Glomeruli were incubated without (control) or with added serotonin as described in the Methods, except that 0.5 mM MIX was not included in the incubation mixture. Values represent the mean ± SEM.

[b] N denotes number of experiments.

[c] Values are significantly different from control (without addition of serotonin) and/or Δ% change from control is significant at $P < 0.05$ or higher level of significance (paired t test).

Table 4. Effects of antagonists on serotonin and histamine stimulation of cAMP in glomeruli

Additions[a]	Increase in cAMP[b] *pmoles/mg protein*	Relative inhibition[c] Δ%
Serotonin (N = 7)	+260.1 ± 47.8[d]	
Serotonin + methysergide (N=7)	+ 15.0 ± 8.1	−94 ± 4[d]
Serotonin (N = 6)	+253.8 ± 52.8[d]	
Serontonin + cinanserine (N = 6)	+ 36.4 ± 16.1	−87 ± 7[d]
Serotonin (N = 5)	+247.5 ± 64.2[d]	
Serotonin + metiamide (N = 5)	+116.3[e] ± 28.5[d]	−46 ± 13[d]
Histamine (N = 6)	+221.0 ± 45.3[d]	
Histamine + methysergide (N = 6)	+120.2 ± 26.7[d]	−40 ± 10[d]
Histamine (N = 6)	+221.0 ± 45.3[d]	
Histamine + cinanserine (N = 6)	+101.0 ± 26.9[d]	−44 ± 16[d]
Histamine (N = 4)	+199.1 ± 39.78	
Histamine + metiamide (N = 4)	+ 7.4 ± 4.48	−93 ± 3[d]

[a] Both agonists (serotonin, histamine) and antagonists (cinanserine, methysergide, or metiamide) were added in final concentration, 10^{-4} M, in all incubations. N denotes number of experiments. All values represent the mean ± SEM.

[b] Increase in glomerular cAMP is above control levels (without addition of drugs; the mean control cAMP level was 54.2 ± 10.4 pmoles/mg protein).

[c] Relative inhibition is expressed as percent decrease from effect of agonist (serotonin or histamine) alone. Inhibition of serotonin with metiamide is significantly ($P < 0.05$ or better, t test) lower than is inhibition with methysergide or with cinanserine. Inhibition of histamine with methysergide or with cinanserine is significantly ($P < 0.05$ or better) lower than it is with metiamide.

[d] Increases or extent of inhibition is significant at $P < 0.05$ or higher level of significance (paired t test).

of serotonin. They did not prevent, however, but only partially reduced the cAMP accumulation elicited by histamine (Table 4). Conversely, metiamide (an H_2-histamine receptor antagonist) did not prevent, it only diminished, in part, the accumulation of cAMP in response to serotonin, whereas it completely blocked the stimulatory effect of histamine (Table 4).

Discussion

The results of this study provide evidence that serotonin acts on the kidney cortex, and particularly on glomeruli, to increase cellular levels of cAMP. Because no prior information existed to indicate whether serotonin would influence renal cyclic nucleotide metabolism in kidney at all, and because in some nonrenal tissues serotonin was reported to increase cAMP [10–15], and in other tissue types to increase selectively only cGMP [16–20], present experimental conditions were aimed to optimize detection of serotonin's effect on renal cyclic nucleotides in either direction. The short (2 min) incubation period was used to detect not only changes in cAMP but also changes in cGMP, which are usually transient, with early peak and subsequent decline [16, 29]. Moreover, a shorter incubation time diminishes the possibility of inactivation of tested agents by the incubated tissues. In control experiments (data not shown here), we found that the patterns of responses to serotonin were analogous also in 5-min and 15-min incubation periods. Inclusion of 0.5 mM MIX inhibits both cAMP and cGMP catabolism to about the same degree [21, 30] but not completely [31] and thus optimizes conditions to observe increases in cyclic nucleotide concentrations; but, it also permits detection of decreases in cyclic nucleotides, in response to hormonal agents. In our previous study on the glomerular cyclic nucleotide system, both increases (for example, those elicited by histamine, carbamylcholine, and PTH) or decreases (for example, those elicited by angiotensin II) were observed [22]. As shown in Table 3, basal

concentrations of cAMP in glomeruli in the absence of MIX are lower, but the stimulatory effect of serotonin is readily detected. High specificity of anti-cAMP and anti-cGMP antibodies allows simultaneous measurements of both cyclic nucleotides in the same tissue extracts, excluding errors due to cross-reactivity.

From the three major anatomic zones of the kidney, serotonin increases cAMP only in cortical slices (Table 1). Lack of a serotonin effect on cyclic nucleotides in the medulla and papilla is unlikely to be due to general unresponsiveness of these preparations to hormones, because cAMP increased predictably in response to AVP in the same experiments. Thus, these comparisons suggest that serotonin receptors associated with cyclic nucleotide generating systems are either absent in rat renal medulla and papilla or that, unlike in cortex, they are undetectable under present conditions. Since carbamylcholine increases cGMP in cortical slices, a general unresponsiveness of the cGMP system to hormonal agents does not seem to explain the lack of a serotonin effect. Because tissue slices were prepared from kidneys thoroughly perfused to remove blood, the increase of cAMP in response to serotonin is most likely due to an interaction of this agent with receptors in renal cortical tissue.

Inasmuch as hormonal agents could act differently on glomerular and tubular components of the kidney cortex [22], the effect of serotonin was studied further in isolated glomeruli and tubules. The striking cAMP increase in glomeruli in response to serotonin (Table 2) indicates that these subunits of nephrons are particularly rich in serotonin receptors associated with the cAMP generating system. This conclusion is emphasized comparing it with the data on tubules or unfractionated cortical slices where much smaller responses in cAMP were observed under the identical incubation conditions.

With respect to findings in tubules, it should be stressed that whereas glomeruli are a homogenous, well-defined preparation, tubular fraction, as studied in our experiments, is a rather heterogenous mixture of tubular fragments from diverse nephron segments, all present in the cortex. Keeping this possible limitation in mind, and considering also the small responses in cortical slices, we think the finding of a small cAMP response to serotonin in tubules may indicate that tubules contain few serotonin receptors associated with the cAMP-generating system, namely when compared with glomeruli (Table 2). Alternatively, a serotonin-sensitive cAMP system in tubules might be localized in a very limited subsegment of tubular system, as it is the case of adenylate cyclase sensitive to β-adrenergic agents [32]. Finally, a small admixture of cells from interstitium or vasculature in the tubular fraction cannot be completely ruled out. Nevertheless, responsiveness of the present tubular preparation to hormones in general is evidenced by the many-fold cAMP increase by PTH, and cGMP stimulation by carbamylcholine.

Serotonin not only caused a several-fold increase in glomerular cAMP at maximal stimulatory doses (10^{-5} to 10^{-4} M), but the molar concentrations of serotonin required for half-maximum stimulation (3×10^{-7} M) seem to lower even that of histamine [22], suggesting high affinity of glomerular receptors for serotonin. Observation that the serotonin antagonists methysergide and cinanserine [33] blocked completely stimulation of glomerular cAMP by serotonin, but only in part reduced cAMP stimulation by histamine, suggests indirectly that serotonin acts in glomeruli on sites different from the H_2-histamine receptor (Table 4). This notion is further supported by the finding that metiamide, an H_2-histamine receptor antagonist [34], completely blocked the effect of histamine (Table 4) [22] and only partially blunted the response to serotonin (Table 4). It is not surprising that the specificity of inhibition of hormonal responses in glomerular cAMP by anti-serotonin drugs and the H_2-blocking agent is not absolute. The serotonin antagonists methysergide and cinanserine are known from other tissue systems to inhibit, in addition to their well-known anti-serotonin effects, to a various degree also action of other biogenic amines [33]. It also was reported recently that derivatives of D-lysergic acid, a potent serotonin antagonist, strongly inhibit an H_2-receptor-associated brain adenylate cyclase [35]. Metiamide in higher concentrations may have a non-specific inhibitory effect on adenylate cyclase [36]; some common structural characteristics of D-lysergic acid and H_2-antihistaminics [35] also suggest the possible interaction of metiamide with serotonin receptors in glomeruli. In any case, this partial cross-reactivity of serotonin blocking agents and H_2-antihistaminics should be taken into consideration in the interpretation of studies in vivo, when the above-mentioned or analogous antagonistic drugs are used as experimental tools to block the effects of endogenous serotonin and histamine on the kidney. Serotonin differs from histamine in its effect on glomeruli also in another aspect: unlike histamine, serotonin selectively stimulates cAMP without detectable in-

fluence on cGMP. This lack of cGMP response occurs in the same preparations of glomeruli in which histamine and carbamylcholine elevate cGMP.

It remains to be analyzed with use of other methodologies in which cell types in glomeruli serotonin increases cAMP. In nonrenal tissues, serotonin was reported to increase selectively cGMP but not cAMP in cells with prominent contractile function [16–18] and to increase cAMP in intimal endothelial [12] and epithelial [14] cell elements. Such a comparison may surmise, indirectly, that serotonin could act primarily on endothelial and epithelial cell types also in glomeruli.

Possible pathophysiologic implications of the present findings should be considered at least briefly. First, serotonin's interaction with glomeruli, which leads to an increase in cAMP, may be related to the effects of serotonin on glomerular dynamics, such as decrease in GFR [1, 3]. Even more relevant could be serotonin's role as it is released locally from activated platelets in the pathogenesis of glomerulopathies [6–8]. Inflammatory responses to acute immune injury were shown to be modulated by cAMP in several model systems [37–39]. It is conceivable that serotonin released locally in glomeruli from platelets, for example, in immune-complex-mediated glomerulopathies [6–8], could act, through cAMP mediation, to influence the glomerular inflammation. Serotonin in low (10^{-7} to 10^{-6} M) concentrations was shown to stimulate fibroblast proliferation, biosynthesis and maturation of collagen [40]; cAMP was reported to stimulate [41, 42] and/or modulate directionality [43] of collagen biosynthesis. Thus, the sustained high local concentrations of serotonin, originated from platelet aggregates or microthrombi in glomeruli, could be an important factor in the development of basement membrane thickening or other sclerosing processes in glomeruli.

It would be premature to make, from the present observations, far-reaching conclusions about cAMP-mediated renal effects of serotonin. Nevertheless, our findings, which demonstrate the presence of a serotonin-sensitive cAMP generating system in glomeruli, may represent a starting point for future investigations on serotonin's role in glomerular pathophysiology.

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