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J Clin Invest. 1975;55(2):431-435. https://doi.org/10.1172/JCI107948.

Research Article

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The Effect of Serotonin (5-Hydroxytryptamine) and Derivatives on Guanosine 3′, 5′-Monophosphate in Human Monocytes

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ABSTRACT The effects of several putative mediators of inflammatory responses on the cyclic nucleotide content of mononuclear leukocytes from human peripheral blood were investigated. Incubation of mononuclear cells with 100 μM serotonin (a maximally effective concentration) for 5 min caused a five- to eightfold increase in their content of guanosine 3′,5′-monophosphate (cGMP) with no change in adenosine 3′,5′-monophosphate (cAMP). Melatonin and related derivatives of 5-hydroxytryptamine, but not tryptamine itself, were as effective as serotonin. Histamine and bradykinin had no effect on cGMP. Polystyrene beads caused accumulation of cAMP in mononuclear cells but did not alter cGMP. On the basis of experiments in which mononuclear cells were divided into adherent (largely monocyte) and nonadherent (largely lymphocyte) populations, it was concluded that the monocytes accumulate cGMP in response to serotonin. It is suggested that serotonin and cGMP may play a role in monocyte motility or migration.

INTRODUCTION

Guanosine 3′,5′-monophosphate (cGMP)[1] has been implicated in several aspects of the human inflammatory response. It has been reported that cGMP, as well as agents purported to cause intracellular accumulation of cGMP, can increase the release of lysosomal enzymes from polymorphonuclear (PMN) leukocytes (1–3) and can enhance the leukotactic response of human PMN's to a bacterial chemotactic factor (4). Adenosine 3′,5′-monophosphate (cAMP) has apparently opposite effects on these processes (1–4). In studies designed to explore further the role of cGMP in leukocyte function, we have investigated the effects of phagocytosis and of several putative mediators of inflammatory responses on the cGMP and cAMP content of leukocytes from human peripheral blood. As reported below, of all agents tested, only serotonin and related tryptamine derivatives caused accumulation of cGMP. It appears that a population of monocytes is responsive to these amines.

METHODS

Blood from fasting, apparently normal, humans was drawn into plastic syringes containing 0.2 vol of acid citrate-dextrose anticoagulant (NIH formula A). A leukocyte-enriched fraction from human blood anticoagulated with 0.1 volume of 4% sodium citrate was purchased from Universal Therapeutics, Laurel, Md. All procedures were carried out at room temperature with centrifugation at 250 *g*, unless otherwise noted. Whole blood or the leukocyte-enriched fraction was diluted with an equal volume of a solution of dextran, mol wt 254,000, (36 mg/ml in 0.15 M NaCl) in 50-ml polypropylene tubes (Falcon Plastics, Division of BioQuest, Oxnard, Calif.). After 30 min, the supernate was removed and centrifuged 10 min at 500 *g*. Sedimented cells were washed and suspended in 0.15 M NaCl. 3 vol of water were added, and 30 s later isotonicity was restored. Cells were collected by centrifugation for 15 min and suspended in Krebs-Ringer medium containing 15 mM Tris buffer (final pH 7.4). Four such preparations, called unfractionated leukocytes, contained 69±6% (mean±SEM) PMN leukocytes and 31±7% mononuclear cells[2]; the ratio of leukocytes to erythrocytes was 1:1.

Received for publication 4 October 1974 and in revised form 2 December 1974.

[1] *Abbreviations used in this paper:* cAMP, adenosine 3′,5′-monophosphate; cGMP, guanosine 3′,5′-monophosphate; PMN, polymorphonuclear.

[2] Zucker-Franklin (5) has found that, although 95% of the cells in the mononuclear cell fractions prepared with Ficoll-Hypaque may on inspection of smears be designated lymphocytes, 44% or more of the cells are able to phagocytose particles and may be functionally defined as monocytes. Thus the percentage of circulating normal human leukocytes usually "counted" as monocytes may be falsely low. We have, therefore, reported data from differential counts of Wright's-stained smears in terms of mononuclear and PMN cells.

TABLE I

Effect of Serotonin on cGMP in Leukocytes from Human Peripheral Blood

Exp. no.	Leukocyte preparation	cGMP content: No serotonin	cGMP content: 100 μM serotonin	Cell count: PMN	Cell count: MN
		pmol/10⁷ cells			
1	Unfractionated	1.7±0.8	3.5±0.4	89	11
	Mononuclear-enriched	5.8±0.8	18±0.6	7	93
	PMN-enriched	1.4±0.1	2.0±0.3	100	0
2	Mononuclear-enriched	6.5±0.9	64±11.2	6	94
	Lymphocyte-enriched	1.6±0.3	1.7±0.4	3	97

The PMN-enriched fraction in exp. 1 was prepared from the infranate after Ficoll-Hypaque separation with dextran sedimentation. In exp. 2 the lymphocytes were prepared from the mononuclear enriched fraction with a glass bead-nylon wool column (10). Cells were incubated with additions as indicated for 5 min. Data are reported as the mean of values from duplicate incubations±one-half the range. MN, mononuclear leukocytes.

Mononuclear cell fractions were prepared by a modification of the method of Böyum (6). Whole blood or the leukocyte-enriched fraction was diluted with 2 vol Hanks' solution from which $CaCl_2$ and $MgCl_2$ were omitted. After 10 min at room temperature, samples (40 ml) of the cell suspension from which platelet clumps had sedimented were transferred to 50-ml tubes, and 12 ml of a mixture of 9% Ficoll (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.): 33.9% Hypaque (Winthrop Laboratories, New York), 24:10 vol/vol, was layered under the blood. The tubes were centrifuged for 40 min, the supernates were aspirated, and the mononuclear cell layers were decanted. Cells were washed twice with the modified Hanks' solution and suspended in Krebs-Ringer-Tris medium. These preparations, referred to as mononuclear cell-enriched, contained 7±2% PMN leukocytes and 93±2% mononuclear cells (mean±SEM, $n = 11$); the ratio of leukocytes to erythrocytes was 2 to 1. (The number of platelets seen was variable, but effects of serotonin on cGMP were similar even when none were detected.)

Samples of cells (2 ml, 1 to 9×10^7 cells) were distributed in plastic vials and incubated for 10 min at 37°C with shaking. Additions in a volume of 0.4 ml (in Krebs-Ringer-Tris medium unless otherwise stated) were then made. After incubation as indicated, a 2.2-ml sample of cells plus medium from each vial was added to 1.0 ml of cold 10% perchloric acid and homogenized with a Brinkmann Polytron (Brinkmann Instruments, Inc., Westbury, N. Y., 4,000 rpm, 45 s). [^{3}H]cGMP (<1 pmol, 4.47 Ci/mmol, New England Nuclear, Boston, Mass.) was added, and samples were centrifuged at 1,000 *g* for 30 min at 4°C. Supernates were separated from the protein pellets, neutralized (pH 7.0) with KOH, and centrifuged for 15 min (1,000 *g*, 4°C). The supernates were transferred to columns (0.6×3 cm) of AG 1-X8 formate, 100–200 mesh (Bio-Rad Laboratories, Richmond, Calif.), which were washed with 10 ml water followed by 10 ml 0.5 N formic acid. cGMP was eluted with 10 ml 4 N formic acid. Eluates were lyophilized, and samples were taken for assay of cGMP and for determination of recovery of [^{3}H]cGMP (which ranged from 45–65%, with no greater than 10% variation in a single experiment).

For radioimmunoassay of cGMP (7), samples were incubated with labeled antigen and antibody (Collaborative Research, Waltham, Mass.) in 50 mM sodium acetate buffer, pH 6.2, at 4°C. Standards of authentic cGMP contained samples of extracts of incubation medium carried through the purification procedure. After 16–24 h, 0.5 mg of globulin from rabbit serum (Grand Island Biological Co., Grand Island, N. Y.) and ammonium sulfate (final concentration 36 g/100 ml) were added to each sample. After centrifugation (1,000 *g*, 40 min, 4°C), the pellets were washed with the ammonium sulfate solution before assay of ^{125}I in a Packard Autogamma spectrometer (Packard Instrument Co., Inc., Edison, N. J.). Of the apparent cGMP in tissue extracts from several representative experiments, 90–99% was hydrolyzed by phosphodiesterase (Sigma Chemical Co., Inc., St. Louis, Mo.). As much as 3.3 nmol of cAMP failed to react in the cGMP assay.

For determination of cAMP, incubations were terminated by adding 0.5 ml of 30% trichloroacetic acid and 0.2 pmol [^{3}H]cAMP (20 Ci/mmol, New England Nuclear) to 2.2-ml samples of cells plus medium. After homogenization (Polytron), cAMP was isolated and assayed by the method of Gilman (8), as previously described (9). Because cyclic nucleotide concentrations sometimes varied considerably with different preparations of cells, in many instances data from individual experiments, each replicated two or more times, are reported.

Serotonin creatinine sulfate, tryptamine hydrochloride and other tryptamine derivatives, and dextran were purchased from Sigma; and histamine dihydrochloride was obtained from Schwarz/Mann (Div., Becton, Dickinson & Co., Orangeburg, N. Y.).

RESULTS

In unfractionated leukocytes incubated with 100 μM serotonin for 5 min, the cGMP content was increased 6.0±2.2 pmol/10⁷ cells, from a basal level of 1.3±0.4 (mean±SEM, $n = 7$). When responses of different populations of cells prepared from a single sample of blood were compared, the effects of serotonin were clearly larger with mononuclear cell-enriched fractions than with unfractionated leukocytes. In exp. 1, Table I, if all of the accumulation of cGMP induced by serotonin occurred in mononuclear cells, it can be calculated that the increase was 13.1 pmol/10⁷ mononuclear cells in the unfractionated preparation and 16.4 pmol/10⁷ mononuclear cells in the mononuclear cell-enriched fraction. Thus, all of the increment produced by serotonin in the unfractionated leukocytes could be accounted for by response of the mononuclear cells. The cGMP content of the PMN cell-enriched fractions was increased little, if at all, by serotonin. (The unfractionated leukocytes and the PMN cell-enriched fraction were prepared with dextran, which might have affected their ability to respond to serotonin, but exposure of mononuclear cell-enriched preparations to dextran did not significantly alter their responsiveness to serotonin.)

In preparations of nonadherent cells (functionally lymphocytes) separated from mononuclear cell-enriched fractions with a glass bead-nylon wool column (10), serotonin had little or no effect on cGMP content (e.g., exp. 2, Table I). When mononuclear cells were sepa-

TABLE II

Effect of Serotonin on cGMP in Monocytes

Leukocyte preparation	Serotonin 100 μM	cGMP content	Cell count* M	Cell count* L
		pmol/10⁷ cells		
Adherent	0	1.4±0.4	86	14
	+	18±1.8		
Nonadherent	0	0.3±0.1	11	89
	+	3.8±0.1		

Samples of suspensions of mononuclear cell-enriched fractions in RPMI 160 medium (5 ml containing 2.5 × 10^7 cells) were transferred to plastic dishes (100 mm diam, Falcon Plastics) and incubated at 37° in an atmosphere of water-saturated 95% air-5% CO_2. After 24 h the dishes were chilled, the nonadherent cells decanted, the dishes washed twice with cold Hanks' balanced salt solution, and then incubated with warm Hanks' at 37° for 30 min. The Hanks' medium was then replaced with Krebs-Ringer Tris for 10 min, and the cells were then incubated with or without serotonin for 5 min. Nonadherent cells were harvested by centrifugation and suspended in Krebs-Ringer-Tris medium before incubation for 5 min with or without serotonin. Data are presented as in Table I.

* For counting, adherent cells were gently removed from dishes with a rubber policeman. Monocytes (M) were identified by staining after incubation in a solution containing 0.05% neutral red. Lymphocytes (L) did not take up neutral red. Cell viability, as evidenced by exclusion of trypan blue, was about 90% in both types of preparations.

rated as described in Table II, the cGMP content and the effect of serotonin on it were considerably greater in the adherent monocyte fraction than in the nonadherent cells. On the basis of the number of monocytes present and if the percentage of viable cells (as indexed by exclusion of trypan blue) was the same for lymphocytes and monocytes, we calculate that the observed effect of serotonin on the cGMP content of the nonadherent cell fraction could be completely accounted for by the monocytes present.

In eight mononuclear cell-enriched preparations, exposure to 100 μM serotonin, a maximally effective concentration (Fig. 1), for 5 min increased the cGMP content by 40.9±10.2 pmol/10^7 cells from a basal level of 8.0±2.5 (mean±SEM). As shown in Fig. 2, the effect of serotonin, evident within 2 min, was maximal at 5 min and was maintained essentially constant between 5 and 30 min of incubation with serotonin.

Melatonin, 50 or 100 μM, caused accumulation of cGMP in the mononuclear cell preparations equivalent to that induced by serotonin in the same concentrations, and both together produced an effect no greater than that of either alone (Table III). At concentrations of 1 or 10 μM, however, melatonin was consistently more

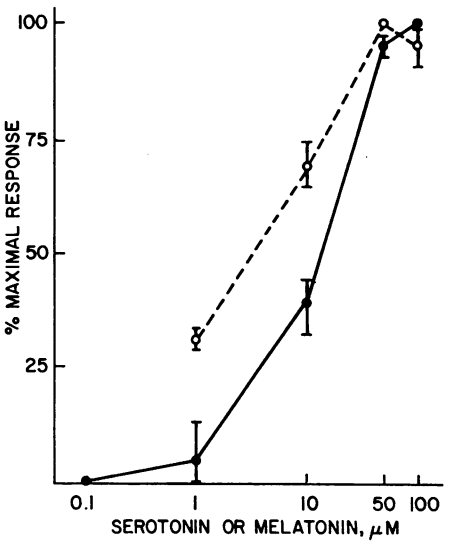

FIGURE 1 Effects of serotonin and melatonin on cGMP in mononuclear cell-enriched preparations. Cells were incubated for 5 min with serotonin (●) or melatonin (○) at the indicated concentrations. The increment in cGMP produced by the addition is expressed as a percentage of the maximal effect produced by each agent. The maximal increase produced by serotonin was 54 pmol, and (in another experiment) by melatonin 89 pmol. Each point represents the value from one incubation or the mean of values from duplicate incubations with the indicated range.

effective than the same amount of serotonin (Fig. 1). *N*-Acetyl 5-hydroxytryptamine, 5-methoxytryptamine, and 5-hydroxymelatonin at concentrations of 100 μM

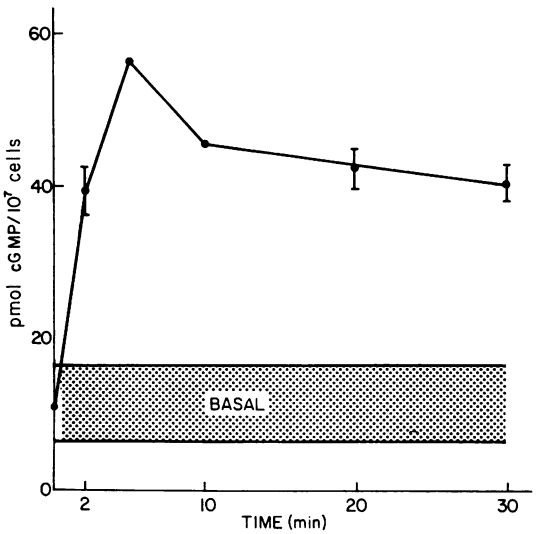

FIGURE 2 Effect of serotonin on cGMP content of mononuclear cell-enriched preparations. Cells were incubated for the indicated time with 50 μM serotonin (●). Each point represents the value from a single incubation or the mean of values from duplicate incubations with the indicated range. The shaded area indicates the mean±SE of values from four samples of cells incubated without serotonin for 2, 5, 10, or 30 min.

TABLE III

Effects of Serotonin and Related Compounds on cGMP in Mononuclear Cells

Exp.	Additions	cGMP content
		pmol/10⁷ cells
1	None	4.9±2.3
	Ethanol, 0.5%	4.7±0.6
	Tryptamine	8.2±0.7
	5-Hydroxytryptamine (serotonin)	39±4.7
	5-Methoxytryptamine*	48±21
	N-acetyl 5-hydroxytryptamine*	50±0.9
	N-acetyl 5-methoxytryptamine (melatonin)*	40±2.5
	6-Hydroxy melatonin*	34±4.7
	Serotonin plus melatonin	42±0.3
2	None	4.5±1.3
	Serotonin	34
	Melatonin*	36±2.6
	Bradykinin, 22 μM	7.5
	Histamine, 0.1 mM	6.9±1.3
	Beads, ca. 10^{10}/ml	3.8±0.4

Mononuclear cell-enriched fractions were incubated for 5 min with additions as indicated. The concentration of tryptamine and of its derivatives was 100 μM. Data are reported as in Table I. Polystyrene beads (1.1 μm diam) were purchased from Dow Chemical Co. (Midland, Mich.). In another experiment, 1 μM histamine had no effect on cGMP.

* 0.5% ethanol was present in the medium.

were as effective as serotonin or melatonin, but tryptamine was ineffective (Table III, exp. 1).

Serotonin (5-hydroxytryptamine) and its derivatives were the only agents found that consistently increased the cGMP content of mononuclear cell-enriched preparations when present in the medium for 5 min. Phytohemagglutinin (PHA-MR 69, Wallace Diagnostic Reagents, div. Burroughs-Wellcome, Research Triangle Park, N. C.), 3 μg/ml, was without effect in our studies although it has been reported (11, 12) to cause accumulation of cGMP in "lymphocytes" prepared by a Ficoll-Hypaque procedure. Histamine and bradykinin (Table III, exp. 2) as well as lysylbradykinin and methionyl lysylbradykinin were also ineffective. In all experiments, cells that failed to respond to other stimuli responded normally to serotonin.

TABLE IV

Effects of Serotonin and Polystyrene Beads on cAMP in Human Leukocytes

Exp.	Leukocyte preparation	Additions	cAMP content
			pmol cAMP/10⁷ cells
1	Unfractionated	None	48±13
		Serotonin, 0.1 mM	38±3
		Theophylline, 1 mM	36±2
		Serotonin, 0.1 mM +theophylline, 1 mM	47±2
		Beads, ca. 10^{10}/ml	192±15
	MN cell-enriched	None	81±7
		Serotonin, 0.1 mM	90±8
		Beads, ca. 10^{10}/ml	808±83

Cells were incubated with additions as indicated for 5 min. Data are presented as in Table I.

As previously reported (13), incubation of unfractionated peripheral blood leukocytes with polystyrene beads markedly increased their cAMP content (Table IV). In earlier studies employing different methods of cell separation it was shown that the response occurred in a population of mononuclear cells (9). As shown in Table IV, the mononuclear cell-enriched fractions prepared with Ficoll-Hypaque exhibited a much greater rise in cAMP content after exposure to beads than did the unfractionated leukocytes. In neither preparation, however, was there any demonstrable effect of the beads on cGMP (Table III, exp. 2). Serotonin did not alter cAMP in unfractionated or mononuclear cell-enriched preparations (Table IV).

DISCUSSION

It has been reported that serotonin can increase the cGMP content of uterine smooth muscle (12). Serotonin also caused accumulation of cGMP in human umbilical artery.[3] No effects of melatonin on cGMP in any tissue have been described. From the experiments reported here it may be concluded that serotonin, melatonin, and certain of their derivatives can induce accumulation of cGMP but not cAMP in the adherent mononuclear cells from human peripheral blood, i.e., the circulating monocytes or macrophages.

Of the several putative mediators of the human inflammatory response tested, only serotonin (and other derivatives of 5-hydroxytryptamine) increased the cGMP content of mononuclear preparations. The role of these amines and of cGMP in monocyte function remains to be elucidated. Estensen, Hill, Quie, Hogan, and Goldberg (4) reported, however, that 8-bromo cGMP and cholinergic agonists can enhance movement of PMN leukocytes, and in preliminary experiments it has been found that serotonin can enhance the movement of monocytes in response to endotoxin-treated serum whereas histamine and bradykinin (which had no demonstrable effect on cGMP) were without effect.[4] Current studies are designed to explore further the relationship between cGMP metabolism and monocyte function, particularly chemotaxis and cell motility.

[3] Clyman, R. I., J. A. Sandler, V. C. Manganiello, and M. Vaughan. Unpublished observations.

[4] Sandler, J., and J. Gallin. Unpublished observations.

ACKNOWLEDGMENTS

The authors thank Drs. Charles Dinarello, Charles Kirkpatrick, Joost Oppenheim, and Mr. Stanley Ward for advice; Dr. Kenneth Foon for column purification of lymphocytes; and Mrs. Betty Hom and Mr. Terrill K. Smith for excellent technical assistance.

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