

EFFECTS OF SEROTONIN, CARBAMYLCHOLINE, AND ASCORBIC ACID ON LEUKOCYTE CYCLIC GMP AND CHEMOTAXIS

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Estensen et al. (7) found that certain agents, e.g., carbamylcholine, which may raise the intracellular concentration of 3',5'-guanosine monophosphate (cyclic GMP), stimulated polymorphonuclear leukocyte (PMN) chemotaxis, while Goetzl et al. (10) showed that ascorbic acid enhanced movement of monocytes and of PMN. In this paper we show that ascorbic acid and carbamylcholine, like serotonin (12), can cause accumulation of cyclic GMP in human monocytes and that all stimulate leukotaxis of these cells. Cholinergic stimulation

increases cyclic GMP in several tissues (11). Serotonin elevates cyclic GMP in the uterus (11) and human umbilical artery (6) as well as in monocytes, but no effects of ascorbic acid on cyclic GMP in leukocytes have been previously recorded.

MATERIALS AND METHODS

Leukocyte fractions were prepared by dextran sedimentation (12) (PMN) or Ficoll-Hypaque separation (Ficoll, Pharmacia Fine Chemicals Inc., Piscataway, N. J.) (3) (mononuclear cells) from normal human blood as previ-

ously described (12). Chemotactic factors were obtained by activation of complement in fresh human serum with endotoxin (*Escherichia coli* 0127:B8 lipopolysaccharide B, Difco Laboratories, Detroit, Mich.) (5). Mononuclear cell chemotaxis was assayed as described by Snyderman et al. (13), using a 5- μ m Nucleopore filter (Wallabs, Inc., San Rafael, Calif.). In this assay, chemotaxis is expressed as the mean number of cells per high power field collected on the lower surface of the filters. PMN chemotaxis, measured using ^{51}Cr (Amersham/Searle Corp., Arlington Heights, Ill.)-labeled PMN and a modified Boyden chamber with two 5- μ m micropore filters (Millipore Corp., Bedford, Mass.), is reported as corrected counts per minute lower filter (8). In both the mononuclear cell and PMN chemotaxis assays, cell suspensions with or without additions (e.g., serotonin or ascorbic acid) were added to one side of the chamber, and buffer, with or without activated serum, to the other. In some experiments, the effects of serotonin, ascorbic acid, or carbamylcholine on spontaneous (random) migration were evaluated using the same chemotactic chambers but substituting Hanks' buffer as the stimulus (2, 9). For both PMN and mononuclear cell migration studies, four replicate chambers were used for each experimental condition. None of the agents tested, when added to the attractant side alone, exhibited chemotactic activity for mononuclear cells or PMN. The means of different experiments were compared, using the one-tailed paired Student's *t*-test.

For measurement of cyclic GMP content, samples of

cells (2 ml, $1-9 \times 10^7$ cells) were distributed to plastic vials and incubated for 10 min at 37°C with shaking. Additions in a volume of 0.4 ml (of 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 10% perchloric acid and homogenized with a Brinkmann Polytron (Brinkmann Instruments, Inc., Westbury, N. Y.) (4,000 rpm, 45 s). [^3H]cyclic GMP (<1 pmol, 4.47 Ci/mmol, New England Nuclear, Boston, Mass.) was added, and samples were purified and assayed for cyclic GMP as previously described (12). None of the agents tested, when purified as experimental samples, had any effect on the cyclic GMP assay. All incubations were carried out in duplicate.

RESULTS AND DISCUSSION

In the mononuclear cell chemotaxis assay, accumulation of cells on the lower surface of the filter was relatively slow for the first 30-45 min, and no effect of serotonin was observed. The rate of accumulation of mononuclear cells increased sharply after 45 min, and the increase was greater in the presence of 10 μM serotonin than in its absence. As shown in Table I, 10 μM serotonin, which did not significantly affect random migration, caused a marked increase in the number of mononuclear cells accumulated on the lower surface of the fil-

TABLE I
Effects of Serotonin on Mononuclear Cell Migration

Exp. no.	Random migration (0.05 ml buffer)			Chemotaxis (0.05 ml activated serum)		
	No serotonin	10 μM serotonin	Δ	No serotonin	10 μM serotonin	Δ
	cells/high power field			cells/high power field		
1	4 \pm 1	20 \pm 5	+16			
2	3 \pm 1	5 \pm 4	+2			
3	10 \pm 2	7 \pm 1	-3			
4	4 \pm 1	9 \pm 1	+5	26 \pm 3	38 \pm 9	+12
5*	12 \pm 2	11 \pm 3	-1	54 \pm 8	72 \pm 5	+18
6				49 \pm 4	69 \pm 5	+20
7				10 \pm 3	69 \pm 10	+59
8*				48 \pm 4	110 \pm 19	+62
9*				25 \pm 1	38 \pm 8	+13
10				73 \pm 8	94 \pm 16	+21
11				66 \pm 8	107 \pm 8	+41
Mean	7	11	+4	44	75	+31
SE	1.8	2.6	4	7	9.5	7.2

Data are presented as mean \pm SE of quadruplicate samples. Assays were carried out for 90 min with or without 10 μM serotonin present in the cell suspension.

* In these experiments, the effect of 50 μM serotonin on chemotaxis was -14 ± 3.7 mononuclear cells/high power field (mean \pm SE).

ters in 90 min when the chemotactic stimulus was present ($P < 0.0025$). In one of these experiments, 5 μM serotonin increased by 63% the number of mononuclear cells accumulated. With 50 μM serotonin, however, no augmentation of the chemotactic response was demonstrable (Table I). Carbamylcholine, an agent previously reported to enhance PMN chemotaxis (7), augmented the chemotactic response of mononuclear cells as well ($P < 0.025$, Table II). We also confirmed the reports of Goetzl et al. that ascorbic acid enhanced the chemotactic responsiveness of mononuclear cells, but because of the small number of experiments and the variability in magnitude of the responses, the difference between the ascorbate-treated cells and control was significant only at the 10% level. Bradykinin (22 μM) and histamine (10 or 100 μM) did not enhance chemotaxis.

In the ^{51}Cr radioassay for PMN chemotaxis, the rate of accumulation of cells in the lower filter was low for 60–90 min and then increased sharply. As shown in Table III, in a 3-h assay period, 10 μM serotonin significantly increased the chemo-

tactic response of these cells. The effect of serotonin when 0.05 ml of activated serum was used as the attractant tended to be greater than when 0.1 ml of serum was present. In the presence of 0.05 ml of activated serum, 5 mM ascorbic acid also enhanced PMN chemotaxis.

In earlier studies (12), serotonin induced a rapid increase in the cyclic GMP content of monocytes, which reached a maximum within 2 min and remained elevated for at least 30 min. After incubation for 90 min with 10 μM serotonin, the cyclic GMP content of mononuclear cells was about twice the basal level. In 5-min incubations, 5 mM ascorbic acid dramatically increased cyclic GMP to levels much higher than those observed with serotonin, whereas the effect of 10 nM carbamylcholine was smaller than that of serotonin (Table IV). Even 50 μM ascorbic acid increased the cyclic GMP content of mononuclear cells about 200% (Fig. 1). No effects of any of these agents on the cyclic GMP content of PMN were demonstrable. The rise in cyclic GMP induced by ascorbic acid (or by serotonin [12]) was accompanied by no change in adenosine 3',5'-monophosphate. We have shown that the increase in cyclic GMP produced by serotonin in mononuclear cell fractions is attributable to the response of the adherent monocytes in these preparations (12), and in similar experiments it was found to be the monocytes, rather than the nonadherent lymphocytes, that accumulated cyclic GMP in response to ascorbic acid (data not shown).

Thus, in the human monocytes, ascorbic acid, carbamylcholine, and serotonin, in concentrations that enhanced chemotactic responsiveness, caused

TABLE II
Effect of Serotonin, Carbamylcholine, and Ascorbic Acid on Mononuclear Cell Chemotaxis

Agent added	Chemotaxis cells/high power field
None	59 \pm 7
Serotonin, 10 μM	76 \pm 9
Carbamylcholine, 1 nM	75 \pm 7
Ascorbic acid, 5 mM	85 \pm 18

Data are presented as mean \pm SE of three experiments in which all agents were simultaneously tested.

TABLE III
Effect of Serotonin on PMN Chemotaxis

Acti- vated serum	No. of exp	Chemotaxis corrected counts per minute	
		No serotonin	Δ due to serotonin
0.05 ml	4	2,244 \pm 617	+1,210 \pm 226*
0.1 ml	4	3,213 \pm 770	+781 \pm 165*

Assays were carried out for 3 h with or without 10 μM serotonin present in the cell suspension. Data are presented as mean \pm SE for the indicated number of experiments.

* $P < 0.01$.

TABLE IV
Effects of Serotonin, Carbamylcholine, and Ascorbic Acid on Cyclic GMP in Mononuclear Cells

Addition	No. of exp	Δ Cyclic GMP content* %
Serotonin, 10 μM	4	+263 \pm 60
Carbamylcholine, 10 nM	4	+61 \pm 23
Ascorbic acid, 5 mM	8	+1636 \pm 203

Cells were incubated for 5 min with or without additions as indicated. Data from 14 different cell preparations are represented. In these the mean basal content of cyclic GMP was 2.1 \pm 0.1 pmol/ 10^7 cells (mean \pm SE).

* Mean \pm SE of percentage changes caused by the addition in the indicated number of experiments.

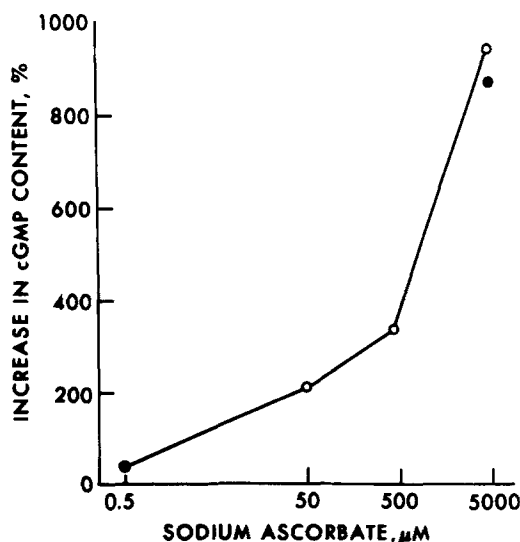


FIGURE 1 Effect of ascorbic acid on cyclic GMP (cGMP) content of mononuclear cells. Cells were incubated with or without ascorbic acid for 5 min at 37°C. Data (means of duplicate incubations) from two different experiments (●, ○) are presented.

accumulation of cyclic GMP, whereas bradykinin and histamine did not enhance chemotaxis or increase cyclic GMP. Only with concentrations of serotonin $> 10 \mu\text{M}$ was there a lack of correlation between increased cyclic GMP and enhanced chemotaxis. Maximal effects of serotonin on cyclic GMP in monocytes were produced with concentrations of 50–100 μM , but effects on chemotaxis were maximal with 10 μM serotonin, and higher concentrations failed to stimulate and even decreased cell movement. This phenomenon has previously been seen in PMN in the presence of the calcium ionophore A23187 which in low concentrations enhances chemotaxis and in higher concentrations is inhibitory (1). Recently, Clark et al. have shown that histamine, too, has a biphasic effect on eosinophil chemotaxis (4). It is conceivable that the inhibitory effect of high concentrations of these agents is exerted at steps subsequent to the accumulation of cyclic nucleotides.

In PMN, on the other hand, although serotonin and, as previously reported (10), ascorbic acid increased chemotaxis, they did not demonstrably alter cyclic GMP. The failure to demonstrate increases in the cyclic GMP content of PMN on exposure to ascorbic acid or serotonin in concentrations that stimulated chemotaxis is not, of course, inconsistent with the suggestion of Estensen et al. (7) that elevation of intracellular

cyclic GMP enhances cell movement. It may be that changes in cyclic GMP too small to be detected in our assay are sufficient to influence PMN function. Alternatively, cyclic GMP elevation may not be a prerequisite for enhanced cell movement, and agents as divergent as serotonin, carbamylcholine, and ascorbic acid may influence chemotaxis through other as yet undefined mechanisms.

SUMMARY

Serotonin, ascorbic acid, and carbamylcholine enhanced the chemotactic responsiveness of human monocytes to endotoxin-treated serum. These agents caused significant accumulation of cyclic GMP in monocytes. PMN leukocyte chemotaxis was also enhanced by these agents although significant increases in cyclic GMP were not demonstrated.

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