MOLECULAR PHARMACOLOGY, 16, 805–812

The Interaction of Colchicine With Hormone-Sensitive Adenylate Cyclase in Human Leukocytes

STEPHEN A. RUDOLPH,[1] LINDA R. HEGSTRAND,[2] PAUL GREENGARD, AND STEPHEN E. MALAWISTA

Departments of Pharmacology and Medicine, Yale University School of Medicine, New Haven, CT 06510

(Received February 5, 1979)
(Accepted July 9, 1979)

SUMMARY

RUDOLPH, S. A., L. R. HEGSTRAND, P. GREENGARD, AND S. E. MALAWISTA: The interaction of colchicine with hormone-sensitive adenylate cyclase in human leukocytes. *Mol. Pharmacol.* **16**: 805–812 (1979).

A histamine-sensitive adenylate cyclase was found in human leukocytes and appears to interact with the microtubule system of these cells. Colchicine markedly potentiated the effects of IBMX, histamine, or both on increasing cyclic AMP levels in intact leukocytes. Preincubation with vinblastine or podophyllotoxin caused effects similar to those of colchicine, while lumicolchicine and cytochalasin B were ineffective. Preincubation with colchicine did not significantly affect the concentration of histamine at which half-maximal increases in cyclic AMP levels were attained (5 μM). Membranes prepared from leukocyte homogenates had a basal adenylate cyclase activity of about 10 pmole/min/mg protein; histamine (10^{-4} M) caused a more than three-fold increase in this activity. The effects of colchicine that were observed with intact cells were not found in the membrane preparation, whether the membranes were prepared from colchicine-treated cells or whether colchicine was added directly to the adenylate cyclase assay mixture. The effects of histamine in both intact cells and cell membranes were blocked by the H_2 antagonist metiamide, but not by the H_1 antagonist mepyramine. The interaction of histamine with two other hormones that activate leukocyte adenylate cyclase *via* different receptors, isoproterenol and prostaglandin E_1 (PGE_1), was also investigated. The effects of all three agonists were not additive with respect to cyclic AMP levels in intact cells, whether in the absence or presence of colchicine, or with respect to adenylate cyclase activity in membrane preparations. Cells enriched for polymorphonuclear or mononuclear leukocytes exhibited responses to histamine, isoproterenol or PGE_1, and to colchicine, similar to those seen in the unfractionated leukocyte preparations. The results suggest that interference by colchicine with the organization of cytoplasmic microtubules increases leukocyte adenylate cyclase activity and renders the cells more sensitive to histamine stimulation, without markedly altering the affinity of the histamine receptor. Effects of colchicine were not observed in cell-free preparations, presumably because the interaction between cytoplasmic microtubules and membranes depends upon the integrity of the cell.

Supported in part by grants from the NIH (MH-17387, AM-10493, AM-19742, AM-05639 and AM-07107), the NSF (NS-08440), the Arthritis Foundation, the Kroc Foundation, and the McKnight Foundation.

[1] Present Address: Department of Pharmacology, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

[2] Present Address: Waisman Center, Neurochemistry Section, University of Wisconsin, Madison, WI 53706.

0026-895X/79/060805-08$02.00/0
Copyright © 1979 by Academic Press, Inc.
All rights of reproduction in any form reserved.

The non-additivity of the hormone effects suggests that histamine, isoproterenol, and PGE_1 may all compete for activation of the same molecules of adenylate cyclase.

INTRODUCTION

It has recently been shown that colchicine, vinblastine, and other drugs inhibiting microtubule assembly raise cyclic AMP levels in human leukocytes and markedly potentiate hormonal effects on cyclic AMP production (1). It was suggested that cytoplasmic microtubules might inhibit lateral mobility of the components of the membrane-bound receptor-adenylate cyclase system in a manner similar to that observed for lectin-receptor complexes (2–4), thus limiting the expression of adenylate cyclase activity. The effects of colchicine and related drugs on cyclic AMP production could then be explained on the basis of their interference with the organization of cytoplasmic microtubules and a resultant increase in the frequency of receptor-adenylate cyclase interactions. This view is supported by the finding of similar dose-response relationships for colchicine effects on the distribution of lectin-receptor complexes on the leukocyte membrane (a measure of the cells' competence to assemble microtubules), and on leukocyte cyclic AMP levels (5).

The present work extends the previous observations to include interactions between histamine and colchicine on cyclic AMP levels in human leukocytes. In addition, adenylate cyclase activity measurements carried out in leukocyte membrane preparations lend further support to the hypothesis that colchicine affects cyclic AMP production through its effect on cytoplasmic microtubules rather than through a direct effect on adenylate cyclase.

The results also show that both the polymorphonuclear and mononuclear cell populations exhibit similar responses to colchicine.

MATERIALS AND METHODS

Leukocyte preparation. Leukocytes were obtained from freshly drawn heparinized blood from adult volunteers by dextran sedimentation and hypotonic lysis of residual red cells as described previously (6). Final cell pellets were suspended in a solution containing 123.5 mM NaCl, 5.0 mM KCl, 0.3 mM $MgCl_2$, 0.5 mM $CaCl_2$, 16 mM sodium phosphate, and 1 unit/ml heparin. The pH was 7.4. The final suspension contained about 2.5×10^7 cells/ml, consisting of 70–80% neutrophils, 20–30% lymphocytes, and 1–4% monocytes and eosinophils. Platelets were rare. All manipulations were carried out in siliconized glass or plastic tubes or flasks. Where indicated, leukocytes were separated into neutrophil-rich and mononuclear cell-rich fractions by centrifugation in Ficoll-Hypaque (7).

Intact cell experiments. Portions of the cell suspension were pre-incubated for 30 min at 37° with colchicine or related drugs. Aliquots (0.2 ml) were then transferred to tubes containing the appropriate amounts of hormone and/or IBMX[3] and incubation was carried out for 2 min. The tubes were then transferred to a boiling water bath for 5 min. After overnight storage at −20°, the samples were thawed and then centrifuged for 10 min at $1200 \times g$. Cyclic AMP was determined in aliquots of the supernatant by the method of Brown *et al.* (8).

Adenylate cyclase assay. Leukocytes were suspended as described above at a concentration of $3–5 \times 10^7$ cells/ml. The cells were homogenized at 4° three times, for 10 seconds each time, with 30 second intervals, using a Tekmar Tissumizer. The homogenate was centrifuged for 10 min at $1000 \times g$. The pellet was discarded and the supernatant was centrifuged for 15 min at $10{,}000 \times g$. The resultant pellet was surface washed with ice-cold buffer containing 0.25 M sucrose and 5 mM Tris-maleate, pH 7.5 and then resuspended in the same buffer to a protein concentration of 1–2 mg/ml (membranes from $\sim 10^8$ cells/ml) using a ground glass homogenizer. The adenylate cyclase reaction mixture contained the following final concentrations (mM): Tris-maleate (pH 7.5), 50; EGTA, 0.5; IBMX, 1;

[3] The abbreviation used is: IBMX, isobutylmethylxanthine.

GTP, 0.03; creatine phosphate, 10; creatine kinase, 4–5 units; 50 μl of membrane suspension; $MgCl_2$, 4; ATP, 0.5; plus test substances as indicated, in a final volume of 500 μl. The enzyme reaction, initiated by the addition of $MgCl_2$ and ATP, was carried out for 12 min at 30° and was terminated by placing the assay tubes in a boiling water bath for 2 min. Aliquots (100 μl) were assayed in triplicate for cyclic AMP by the method of Brown *et al.* (8). Protein was determined by the method of Lowry *et al.* (9).

Experiments were performed at least three times with independent preparations of cells and with substantially similar results. Each table or figure shows the results of a representative experiment.

Lumicolchicine was prepared and assayed as described previously (1, 10). Other drugs were obtained from the following sources: histamine hydrochloride, *dl*-isoproterenol hydrochloride, and colchicine from Sigma; vinblastine (Velban) from Eli Lilly, podophyllotoxin and IBMX from Aldrich; cytochalasin B from ICN. Prostaglandin E_1 was a gift of Dr. J. E. Pike of Upjohn. All chemicals were reagent grade.

RESULTS AND DISCUSSION

The effects of histamine, IBMX, and colchicine on the cyclic AMP levels of human leukocytes are shown in Table 1. Histamine (10^{-4} M) alone did not have a substantial effect on cyclic AMP accumulation. IBMX plus histamine had a synergistic effect, resulting in a more than ten-fold increase in cyclic AMP level. When cells were pre-incubated for 30 min with 10 μM colchicine, basal levels of cyclic AMP remained unchanged, and histamine alone doubled the level of cyclic AMP. Pre-incubation with colchicine greatly potentiated the effect of IBMX plus histamine on cyclic AMP levels.

TABLE 1

Effects of colchicine, histamine, and IBMX on human leukocyte cyclic AMP levels

Cells were pre-incubated for 30 min at 37° in the absence or presence of 10 μM colchicine. Aliquots were then transferred to tubes containing the indicated drugs and incubation was carried out for 2 min. The cells were then treated and cyclic AMP measured as described in the text.

Additions	Cyclic AMP, pmol/10^6 cells[a]	
	Control	Colchicine
None	0.35 ± 0.01	0.32 ±0.04
IBMX, 1 mM	0.64 ± 0.03	1.43 ± 0.17
Histamine, 100 μM	0.45 ± 0.03	0.72 ± 0.09
Histamine + IBMX	3.65 ± 0.15	10.70 ± 0.60

[a] Values shown are the means of triplicate determinations ± SEM.

The dose-dependence of the effect of histamine on cyclic AMP levels is shown in Fig. 1. The lower curve (control cells) shows a more than six-fold increase due to histamine, with a half-maximal effect at about 5×10^{-6} M. The upper curve demonstrates the effect of histamine on colchicine-treated cells, and is half-maximal at about 3×10^{-6} M histamine. The colchicine-treated cells appeared to respond to somewhat lower concentrations of histamine, with a significant effect being observed at 10^{-6} M, while the control cells required 3×10^{-6} M.

Several histamine agonists and antagonists were tested for their effects on leukocyte cyclic AMP levels (Table 2). The H_1-histamine agonist, 2-methyl histamine (50 μM), had only a small effect while the H_2-histamine agonist, 4-methyl histamine (50 μM), was about half as effective as histamine (100 μM) in raising cyclic AMP levels. The H_2 histamine antagonist, metiamide (1 mM), almost completely blocked the effect of histamine, while the H_1 antagonists, promethazine (1 μM) and mepyramine (10 μM), were ineffective. Analogous results were obtained with these compounds in colchicine-treated cells (data not shown). Although detailed dose-response studies were not done with each of the drugs in Table 2, the data suggest that the histamine receptor responsible for increasing leukocyte cyclic AMP levels is an H_2 receptor.

A number of drugs were tested for their ability to produce colchicine-like effects on the histamine-dependent increase in leukocyte cyclic AMP levels (Table 3). Podophyllotoxin, an inhibitor of microtubule assembly that binds to the same site on tubulin as colchicine, also potentiated the effects of histamine and IBMX. Vinblastine, an inhibitor of microtubule assembly that binds to a site on tubulin different

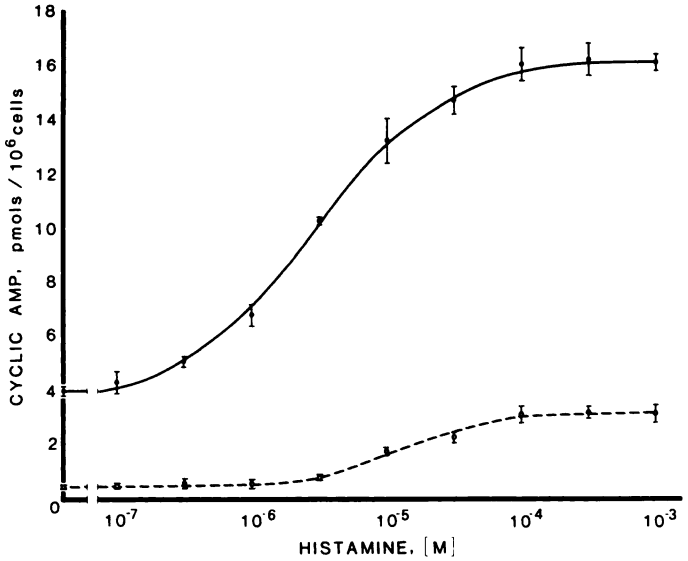

FIG. 1. *Effects of histamine and colchicine on leukocyte cyclic AMP levels*

Cell suspensions were pre-incubated in the absence or presence of 10 μM colchicine for 30 min at 37°. Aliquots were then transferred to tubes containing IBMX (final concentration, 1 mM) and histamine to give the indicated final concentrations. Incubation was carried out for 2 min; the cells were then treated and cyclic AMP measured as described in the text. The points and error bars show the means of triplicate determinations ± SEM. O- - -O, control; ●- - -●, colchicine-treated.

TABLE 2

Effects of histamine receptor agonists and antagonists on human leukocyte cyclic AMP levels

Cells were incubated for 2 min in the presence of 1 mM IBMX plus the indicated drugs. The cells were then treated and cyclic AMP measured as described in the text.

Additions	Cyclic AMP, pmole/10^6 cells[a]
None	0.60 ± 0.05
Histamine, 100 μM	2.39 ± 0.03
2-Methyl histamine, 50 μM	0.93 ± 0.10
4-Methyl histamine, 50 μM	1.76 ± 0.13
Histamine + metiamide (1 mM)	0.85 ± 0.03
Histamine + promethazine (1 μM)	2.46 ± 0.08
Histamine + mepyramine (10 μM)	2.55 ± 0.04

[a] Values shown are the means of triplicate determinations ± SEM.

from the colchicine-binding site, also potentiated the effects of histamine and IBMX. In contrast, lumicolchicine, a photoderivative of colchicine that does not bind to tubulin or inhibit microtubule assembly, and cytochalasin B, a fungal metabolite which interferes with the organization of microfilaments, did not affect leukocyte cyclic AMP levels.

These data on the actions of histamine, IBMX, and colchicine on leukocyte cyclic AMP levels are similar to previous data (1) obtained using *dl*-isoproterenol and PGE_1 as agonists. We proposed in that report that cytoplasmic microtubules may limit the hormonal activation of adenylate cyclase by restricting the lateral mobility of membrane proteins. Colchicine and other inhibitors of microtubule assembly would then potentiate hormonal effects on cyclic AMP levels by causing the disappearance of cytoplasmic microtubules with a concomitant increase in interaction among components of the hormone-sensitive adenylate cyclase system (see below). The present results lend further support to these ideas by showing that drugs acting on a third kind of hormone receptor (the histamine H_2 receptor), independent of the β-adrenergic and PGE_1 receptors, also interact synergistically with colchicine and related drugs.

If the effects of colchicine on hormone-dependent changes in leukocyte cyclic AMP levels are indeed a result of interference with interactions between the cell membrane and cytoplasmic structural elements, then one would not expect to observe these effects in a cell-free system.

Accordingly, we have measured histamine-sensitive adenylate cyclase activity in membrane preparations from human leukocytes. We have found that histamine causes about a threefold increase in adenylate cyclase activity. The histamine dose-dependence of this activity is shown in Fig. 2. It is clear that in the membrane preparation, neither pretreatment with colchicine nor addition of colchicine to the assay mixture had an effect on adenylate cyclase activity at any histamine concentration tested. This is in marked contrast to the data for intact cells, where colchicine was shown to potentiate the effects of both histamine and IBMX. Despite the fact that our data show no direct effect of colchicine on adenylate cyclase activity in membrane preparations, we feel that the effect of colchicine on cyclic AMP levels in intact cells is due to an increase in adenylate cyclase activity rather than a decrease in cyclic AMP degradation. This conclusion is based on the observations that colchicine causes no increase in cyclic AMP level by itself whereas IBMX does, and that the increase due to IBMX is potentiated by colchicine (see Table 1 and ref. 1).

TABLE 3

Effects of various drugs on control and histamine-stimulated cyclic AMP levels in human leukocytes

Cells were pre-incubated for 30 min at 37° in the presence of the indicated drugs. IBMX alone or IBMX plus histamine (final concentrations 1 mM and 100 μM, respectively) were then added to the various tubes and the incubation was carried out for 2 min. The cells were then treated and cyclic AMP measured as described in the text.

Additions	Cyclic AMP pmole/10^6 cells[a]	
	IBMX	Histamine + IBMX
None	0.85 ± 0.07	4.00 ± 0.18
Colchicine, 1 μM	2.66 ± 0.11	14.7 ± 1.2
Podophyllotoxin, 1 μM	1.97 ± 0.01	17.3 ± 0.20
Vinblastine, 1 μM	3.02 ± 0.06	18.3 ± 0.70
Lumicolchicine, 1 μM	0.99 ± 0.06	3.84 ± 0.02
Cytochalasin B, 10 μM	0.84 ± 0.02	4.24 ± 0.20

[a] Values shown are the means of triplicate determinations ± SEM.

The effects of various blockers on the leukocyte histamine-sensitive adenylate cyclase are shown in Table 4. The H_2 antagonists, metiamide (100 μM) and cimetidine

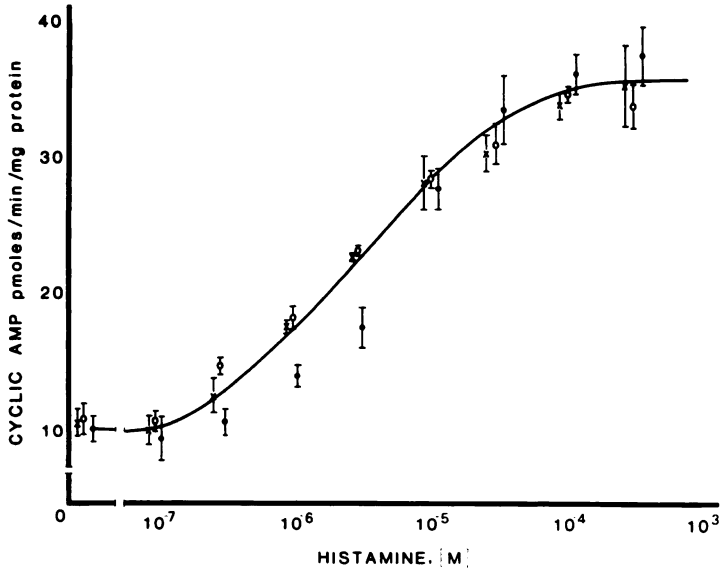

FIG. 2. *Effects of histamine and colchicine on leukocyte membrane adenylate cyclase activity*

Leukocyte membranes were prepared and adenylate cyclase activity was measured with the indicated concentrations of histamine as described in the text. O---O, control; ×---×, cells pre-incubated with 50 μM colchicine for 15 min at 37° prior to homogenization, and colchicine (50 μM) present in the adenylate cyclase assay mixture; ●---●, colchicine (50 μM) present in the adenylate cyclase assay mixture. The points and error bars show the means of six determinations ± SEM.

TABLE 4

Effects of histamine and of histamine receptor antagonists on adenylate cyclase activity in membranes from human leukocytes

Drugs were added to leukocyte membrane preparations and adenylate cyclase activity was measured as described in the text.

Additions	Adenylate cyclase activity,[a]
	(pmols cyclic AMP/min/mg protein)
None	12.5 ± 0.4
Histamine, 100 μM	39.7 ± 0.8
Histamine + metiamide, 100 μM	12.4 ± 0.5
Histamine + cimetidine, 100 μM	12.6 ± 0.7
Histamine + mepyramine, 10 μM	40.1 ± 1.3

[a] Values shown are the means of 6 determinations ± SEM.

TABLE 5

Effects of histamine, dl-isoproterenol, PGE_1, and colchicine on human leukocyte cyclic AMP levels

Cells were pre-incubated for 30 min at 37° in the absence or presence of 10 μM colchicine. Aliquots were then transferred to tubes containing IBMX and the indicated drugs and incubated for 2 min. The cells were then treated and cyclic AMP measured as described in the text. Concentrations of drugs used gave maximal effects and were: IBMX, 1 mM; histamine, 100 μM; *dl*-isoproterenol, 2 μM; PGE_1, 10 μM.

Additions	Cyclic AMP, pmole/10^6 cells[a]	
	Control	Colchicine
None	1.07 ± 0.03	2.66 ± 0.11
Histamine	6.91 ± 0.42	20.1 ± 0.9
dl-Isoproterenol	4.43 ± 0.21	20.1 ± 0.6
PGE_1	6.95 ± 0.10	26.1 ± 3.3
Histamine + *dl*-isoproterenol	5.71 ± 0.29	22.3 ± 1.3
Histamine + PGE_1	7.18 ± 0.21	22.2 ± 0.9
dl-Isoproterenol + PGE_1	7.35 ± 0.18	25.8 ± 2.0
Histamine + *dl*-isoproterenol + PGE_1	7.57 ± 0.27	24.5 ± 3.8

[a] Values shown are the means of triplicate determinations ± SEM.

(100 μM), completely blocked the stimulatory effect of histamine (100 μM), whereas the H_1 antagonist, mepyramine (10 μM), was without effect. These results confirm the conclusion drawn from the intact cell data of Table 2 that the leukocyte histamine receptor is of the H_2 type.

We have also examined the effects of various combinations of hormones and colchicine on cyclic AMP production in both intact leukocytes and leukocyte membranes. The results of these experiments are shown in Tables 5 and 6. The data in Table 5 show that the effects of the three hormones on cyclic AMP levels in any combination are non-additive, both in control and colchicine-treated cells. Current knowledge about hormone-sensitive adenylate cyclase suggests that the receptor and the enzyme are separate, membrane bound entities (11, 12) that interact *via* an associative mechanism. A reasonable interpretation of the data in Table 5 is that each class of receptors can compete for the same pool of adenylate cyclase molecules and can activate all of them (although with somewhat different maximal effect). The data argue against the possibility that colchicine allows interaction of adenylate cyclase molecules, that had previously been associated with only one receptor, with the other receptors. If this were the case, then one would expect additivity of the effects of the three hormones in control cells, but non-additivity in colchicine-treated cells. The data, however, show that this is not the case.

The effects of combinations of histamine, *dl*-isoproterenol, and PGE_1 on leukocyte membrane adenylate cyclase activity are shown in Table 6. As was observed for the intact cells, the effects of the three hormones are non-additive. Since the adenylate cyclase activity of the membrane preparation should not be limited by the availability of ATP, GTP, or some other cytoplasmic factor, the data of Table 6 further support the suggestion that each class of hormone receptor competes for the same pool of adenylate cyclase molecules. The data of Tables 5 and 6 also lead to the conclusion that the three types of hormone-sensitive adenylate cyclases are present in each major class of cell, rather than in separate subpopulations sensitive to different hormones.

Since the crude leukocyte preparation consisted of two major cell types, we ex-

amined the response of cyclic AMP levels to hormones and colchicine in purified neutrophil and mononuclear cell fractions. The results of these experiments are shown in Table 7. Both fractions demonstrated effects similar to those observed in the mixed leukocyte preparation. Basal cyclic AMP levels were less than 1 pmole/10^6 cells and were increased by IBMX. All three hormones, histamine, *dl*-isoproterenol, and PGE_1, gave several-fold stimulation of cyclic AMP levels. In addition, the potentiation of the hormone effects by colchicine was observed in both cell fractions. These data provide evidence that in terms of their interaction with the cell membrane, cytoplasmic microtubules may be functionally similar in neutrophils and in mononuclear cells. Indeed, there is already some evidence for this, in that both cell fractions require colchicine treatment for maximal capping of lectin-receptor complexes to be observed (2–4).

The data presented support the conclusion that colchicine potentiates the effects of hormones on cyclic AMP levels in human leukocytes through its inhibitory effect on microtubule assembly rather than through a direct effect on phosphodiesterase, adenylate cyclase, or the hormone receptor. Other inhibitors of microtubule assembly have similar effects on cyclic AMP levels, even though they are structurally unrelated and affect microtubule assembly by binding to different sites on the tubulin molecule. These drugs are not specific to a particular hormone-sensitive adenylate cyclase, but potentiate the effects of hormones acting at histamine H_2 receptors, β-adrenergic receptors, and PGE_1 receptors. In addition, the effects of colchicine are not observed in membrane preparations, which are free of cytoplasm, but which do respond to all three hormones. As discussed previously (1,

TABLE 6

Effects of histamine, dl-isoproterenol, and PGE_1 on adenylate cyclase activity in membranes from human leukocytes

Adenylate cyclase activity was measured in leukocyte membrane preparations as described in the text in the presence of the indicated drugs. The concentrations used gave maximal effects and were: histamine, 100 μM; *dl*-isoproterenol, 2 μM; PGE_1, 10 μM.

Additions	Adenylate cyclase activity,[a]
	(pmols cAMP/min/mg protein)
None	12.5 ± 0.4
Histamine	39.7 ± 0.8
dl-Isoproterenol	24.4 ± 1.0
PGE_1	24.6 ± 0.9
Histamine + *dl*-isoproterenol	37.3 ± 0.6
Histamine + PGE_1	37.9 ± 2.0
dl-Isoproterenol + PGE_1	27.1 ± 1.1
Histamine + *dl*-isoproterenol + PGE_1	41.0 ± 1.3

[a] Values shown are the means of 6 determinations ± SEM.

TABLE 7

Effects of histamine, dl-isoproterenol, PGE_1, and colchicine on cyclic AMP levels of purified human neutrophils and mononuclear cells

Leukocytes were separated into neutrophil (98%) and mononuclear cell (lymphocytes, 95%) fractions by centrifugation through a Ficoll-Hypaque gradient (6). Cell suspensions were then pre-incubated in the absence or presence of 10 μM colchicine for 30 min. Aliquots were then added to tubes containing the indicated drugs and incubated for 2 min. The cells were then treated and cyclic AMP measured as described in the text. Final concentrations were as follows: IBMX, 1 mM; histamine, 100 μM; *dl*-isoproterenol, 2 μM; PGE_1, 10 μM.

Additions	Cyclic AMP, pmols/10^6 cells[a]			
	Neutrophils		Mononuclear Cells	
	Control	Colchicine	Control	Colchicine
None	0.26 ± 0.04	0.31 ± 0.02	0.84 ± 0.16	1.00 ± 0.11
IBMX	0.81 ± 0.02	1.59 ± 0.05	1.21 ± 0.11	1.42 ± 0.16
IBMX + histamine	3.55 ± 0.21	15.4 ± 0.4	4.42 ± 0.42	13.3 ± 0.7
IBMX + *dl*-isoproterenol	2.93 ± 0.09	14.1 ± 0.5	4.95 ± 0.42	11.6 ± 0.4
IBMX + PGE_1	6.94 ± 0.17	21.3 ± 0.7	11.7 ± 1.3	22.1 ± 1.4

[a] Values shown are the means of triplicate determinations ± SEM.

5), a possible explanation for the effects of colchicine is an increase in the lateral mobility of membrane proteins in cells treated with this drug. The nonadditivity of the effects of the three hormones on cyclic AMP formation in intact cells indicates that the effects of colchicine cannot be attributed solely to an increased interaction between the hormone receptor itself and the catalytic moiety of the adenylate cyclase. However, kinetic considerations indicate that any of several more complex mechanisms involving an increase in the lateral mobility of membrane constituents could account for our observations with colchicine. One such mechanism would be an increased frequency of interaction between a [hormone-hormone receptor-GTP binding protein] complex and the catalytic moiety of the enzyme.

REFERENCES

1. Rudolph, S. A., P. Greengard, and S. E. Malawista. Effects of colchicine on cyclic AMP levels in human leukocytes. *Proc. Natl. Acad. Sci. USA.* **74:** 3404–3408, 1977.
2. Berlin, R. D. and T. E. Ukena. Effect of colchicine and vinblastine on the agglutination of polymorphonuclear leucocytes by Concanavalin A. *Nature New Biol.* **238:**120–122, 1972.
3. Yin, H. H., T. E. Ukena, and R. D. Berlin. Effect of colchicine, colcemid, and vinblastine on the agglutination, by Concanavalin A, of transformed cells. *Science* **178:** 867–868, 1972.
4. Edelman, G. M., I. Yahara, and J. L. Wang. Receptor mobility and receptor-cytoplasmic interactions in lymphocytes. *Proc. Natl. Acad. Sci. USA* **70:** 1442–1446, 1973.
5. Malawista, S. E., J. M. Oliver, and S. A. Rudolph. Microtubules and cyclic AMP levels in human leukocytes: On the order of things. *J. Cell. Biol.* **77:** 881–886, 1978.
6. Malawista, S. E. and P. T. Bodel. The dissociation by colchicine of phagocytosis from increased oxygen consumption in human leukocytes. *J. Clin. Invest.* **46:** 786–796, 1967.
7. Böyum, A. Separation of leucocytes from blood and bone marrow. *Scand. J. Clin. Lab. Invest.* **21** (Suppl.): 97, 1968.
8. Brown, B. L., J. D. M. Albano, R. P. Ekins, A. M. Sgherzi, and W. Tampion. A simple and sensitive saturation assay method for the measurement of adenosine 3′-5′-cyclic monophosphate. *Biochem. J.* **121:** 561–562, 1971.
9. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275, 1951.
10. Wilson, L. and B. Friedkin. The biochemical events of mitosis. I. Synthesis and properties of colchicine labeled with tritium in its acetyl moiety. *Biochemistry* **5:** 2463–2468, 1966.
11. Limbird, L. E. and R. J. Lefkowitz. Resolution of β-adrenergic receptor binding and adenylate cyclase activity by gel exclusion chromatography. *J. Biol. Chem.* **252:** 799–802, 1977.
12. Orly, J. and M. Schramm. Coupling of catecholamine receptor from one cell with adenylate cyclase from another cell by cell fusion. *Proc. Natl. Acad. Sci. USA* **73:** 4410–4414, 1977.