slope of 58 mv predicted by the Nernst equation for a K^+ electrode (shown by the continuous line in Fig. 2). Our results indicate that the receptor potential is mainly due to an increase in membrane permeability to K^+.

It is apparent from Fig. 2 that membrane potential in darkness is not solely determined by permeability to K^+. Figure 1C shows the effect of removing external Na^+ on the resting potential and light response. Replacement of Na^+ with tris, choline, or lithium caused the resting potential to become more hyperpolarized and reduced the amplitude of the light response. However, when saturating flashes were used the absolute potential reached by the peak of the light response was very little affected by complete removal of Na^+. The low value of the membrane potential in the dark and the large hyperpolarization produced by removal of external Na^+ suggest that permeability to Na^+ contributes significantly to resting potential in these cells; that is, compared with other neurons they have a relatively high ratio of Na^+ permeability to K^+ permeability.

It is of interest that a light-evoked hyperpolarization in a nonreceptor cell, the giant neuron of the *Aplysia* abdominal ganglion, is also produced by an increase in permeability to K^+ (*10*). This response has a very long latency and a slow time course, and is believed to result from absorption of light by intracellular pigments (*11*) of a different type than the membrane-bound pigments normally associated with photoreceptor cells. It is possible, however, that in both receptor and nonreceptor cells the effect of illumination is to release a similar intermediate product which initiates the permeability changes that occur in the plasma membrane. As yet the actual sequence of reactions leading to changes in membrane permeability has not been worked out for any photoreceptor system.

This is the first demonstration that a visual receptor potential can be produced by a selective increase in membrane permeability to K^+. In other sensory receptor cells, both visual and nonvisual, where the ionic basis of the response has been studied, the receptor potential has been attributed mainly to changes in Na^+ permeability. Our results, along with the recent finding that the hyperpolarizing mechanoreceptor potential in *Paramecium* is due to an increase in K^+ conductance (*12*), establish that the changes in ionic permeability which produce the receptor potential need not be specific for or even involve Na^+. It is likely that changes in K^+ permeability also underlie the receptor potential of other photoreceptors.

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9 October 1973

Adenosine 3′,5′-Monophosphate: Inhibition of Complement-Mediated Cell Lysis

Abstract. *An increase in adenosine 3′,5′-monophosphate (cyclic AMP) in rat mast cells, achieved by stimulating the cells with prostaglandin E_1, by preventing cyclic AMP breakdown with aminophylline, or by adding exogenous dibutyryl cyclic AMP, prevented complement-mediated cytolysis as assessed by both histamine release and vital dye exclusion. Dibutyryl cyclic AMP also suppressed water-induced osmotic lysis.*

In contrast to the facilitory role of adenosine 3′,5′-monophosphate (cyclic AMP) as a "second messenger" initiating intracellular responses to extracellular stimuli, its role in the antigen-induced secretion of chemical mediators from human peripheral leukocytes, lung, and nasal polyps sensitized with immunoglobulin E (IgE) is suppressive (*1*). Its action in lung is preceded by a calcium-dependent activation of a serine esterase, an energy-dependent step, and a second calcium-dependent stage inhibitable by ethylenediaminetetraacetic acid (*2*). The inverse relationship between intracellular concentrations of cyclic AMP and mediator release has now been directly demonstrated by use of purified rat mast cells challenged with rabbit antiserum directed against rat Fab, a noncytolytic reversed anaphylactic reaction (*3*). Histamine release from rat mast cells may also be immunologically achieved by complement-dependent cytolytic mechanisms, and the role of cyclic AMP in modulating this reaction was investigated.

The interaction of mast cells with rabbit antiserum directed against mast cell–specific antigens forms stable sensitized cells that lyse after addition of normal serum as a source of complement (*4*). Pleural and peritoneal cells from male Sprague-Dawley and ACI rats (Gofmoor Farms, Westboro, Massachusetts) weighing 250 to 300 g were obtained (*5*), and the mast cells were studied either in mixed cell suspensions containing 5 to 10 percent mast cells or after isolation to 90 to 98 percent homogeneity by centrifugation into a solution of Ficoll (Pharmacia, Uppsala, Sweden) (*6*). Rabbit antiserum against rat mast cells (Rab anti-RMC) prepared as described was heated at 56°C for 30 minutes and dialyzed against Tyrode's solution (*4*). Interaction of Rab anti-RMC with either mixed cell suspensions or isolated mast cells—both suspended in Tyrode's 0.1 percent solution (Tyrode's gel) so as to contain 10^5 mast cells in 0.1 ml—for 15 minutes at 37°C, followed by washing, sensitized the cells such that the introduction of rabbit serum (10 percent by

volume) as a complement source induced the release of 15 to 90 percent of the total histamine content within 30 minutes at 37°C. The dilution of antiserum was adjusted from 1:200 to 1:500 for each experiment to achieve approximately 25 to 50 percent histamine release after whole complement was added. Histamine released into the supernatant and residual tissue histamine extracted by boiling the cells for 8 minutes were quantitated by bioassay on the isolated, atropine-treated guinea pig ileum (*7*), and the data were expressed as the net percentage of histamine released. Each result represents the mean of triplicate samples, and individual determinations varied by less than 5 percent of the mean.

That the release of histamine from cells sensitized with Rab anti-RMC involved the terminal components of complement was again established (*4*) by demonstrating that serum derived from rabbits congenitally deficient in the sixth component of complement (C6) failed to induce histamine release unless reconstituted with purified human C6. The addition of normal rabbit serum to mast cells in a mixed cell suspension sensitized with Rab anti-RMC produced a net histamine release of 43 percent. When added alone, C6-deficient serum or purified human C6 (*8*) released no histamine, but when combined to achieve normal serum hemolytic activity, they resulted in 42 percent release.

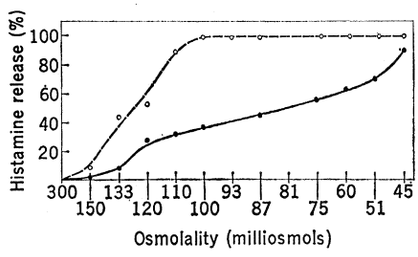

Fig. 1. Capacity of dibutyryl cyclic AMP to inhibit water-induced osmotic lysis of rat mast cells. Mast cells (10^5) in a mixed cell suspension were incubated with $10^{-2}M$ dibutyryl cyclic AMP for 30 minutes at 37°C before addition of varying quantities of distilled water to reduce the osmolality (●). Replicate samples were maintained in Tyrode's gel in parallel before addition of distilled water (○). The osmolality was determined by freezing point depressions.

Prostaglandin E_1 (PGE_1), an activator of adenylate cyclase which increases cyclic AMP concentration in mast cells, produced a dose-dependent inhibition of the capacity of complement to induce histamine release from sensitized cells at PGE_1 concentrations from 5×10^{-4} to $5 \times 10^{-6}M$ (experiment A in Table 1). Aminophylline (10^{-2} to $10^{-3}M$), a competitive inhibitor of cyclic nucleotide phosphodiesterase which has also been demonstrated to increase the cyclic AMP concentration of mast cells (*3*), similarly induced a dose-related inhibition of complement-mediated lysis. Aminophylline and dibutyryl cyclic AMP in combination markedly suppressed histamine release at concentrations that had no effect when used alone.

Incubation of mast cells sensitized with Rab anti-RMC in varying concentrations of dibutyryl cyclic AMP consistently produced a dose-related inhibition of complement-mediated lysis, with an ID_{50} (dose producing 50 percent inhibition) in seven individual experiments ranging from 10^{-2} to $10^{-3}M$. The inhibitory action of exogenous dibutyryl cyclic AMP was evident regardless of the dilution of the antiserum employed, 1 : 200 to 1 : 500; the species of rat from which the mast cells were obtained, Sprague-Dawley or ACI; and the type of mast cell preparation, mixed cell suspensions or purified. Dibutyryl cyclic AMP not only inhibited histamine release but also prevented impaired dye exclusion, a classical test of cell viability (experiment B in Table 1). Inasmuch as the final lytic effect of complement is osmotic lysis secondary to the actions of the terminal complement components on the cell membrane (*9*), the effect of cyclic AMP on the water lysis of mast cells was studied (Fig. 1). Reduction of the osmolality of the incubation medium from 300 to 100 milliosmols induced 100 percent lysis of mast cells, whereas dibutyryl cyclic AMP prevented 100 percent lysis until osmolality was reduced to 45 milliosmols. In data not shown, dibutyryl guanosine 3′,5′-monophosphate (dibutyryl cyclic GMP) had no effect on water lysis.

These experiments indicate that increased intracellular concentrations of cyclic AMP achieved by endogenous production through stimulation with PGE_1 (Table 1), by protection of intracellular cyclic AMP from breakdown by aminophylline, or by the addition of exogenous dibutyryl cyclic AMP (Table 1) prevent complement-mediated cytolysis as assessed by either histamine release or exclusion of vital dye. These results could be attributed to prevention of the insertion of the terminal complement components into the cell membrane (*9*) or of the subsequent osmotic lysis. The observation that dibutyryl cyclic AMP prevents not only the complement-mediated reaction but also water-induced osmotic lysis of mast cells (Fig. 1) favors an effect of this cyclic nucleotide on membrane integrity, a conclusion reached independently in studies in which increased

Table 1. Capacity of increased concentrations of cyclic AMP to inhibit complement-mediated histamine release and cytolysis. For cyclic AMP determinations, 5×10^5 isolated rat mast cells in 0.1 ml were interacted with varying concentrations of PGE_1 for 5 minutes at 37°C, rapidly frozen in a Dry Ice–actone bath, frozen and thawed three times, diluted to 1 ml with distilled water, and boiled for 10 minutes. The solutions were each applied to 0.4 by 2 cm Dowex AG 1 columns (200-400 mesh, formate form, BioRad, Richmond, California) and washed with 10 ml of distilled water; cyclic AMP was eluted with 10 ml of 2*N* formic acid. The eluates were evaporated to dryness and resuspended in 0.5 ml of acetate buffer (50 m*M*, *p*H 4.0); cyclic AMP was quantitated as described (*11*). Results represent the mean of triplicate samples ± standard error of the mean. In histamine release measurements, 10^5 isolated mast cells were sensitized with Rab anti-RMC, incubated with varying quantities of PGE_1 for 5 minutes or with dibutyryl cyclic AMP for 30 minutes at 37°C, and interacted with whole serum complement. The samples containing PGE_1 and the appropriate controls were extracted with three volumes of water-saturated ethyl ether five times before bioassay. Trypan blue was used at a final concentration of 1 percent; 62 to 68 percent of cells either maintained in Tyrode's gel, sensitized with Rab anti-RMC, or treated with normal rabbit serum exhibited the capacity to exclude the dye.

Agent	Concentration (*M*)	Cyclic AMP (picomoles per 10^6 mast cells)	Histamine release (net %)	Trypan blue exclusion (% of cells)
		Experiment A		
PGE_1	0	2.4 ± 0.66	24	
	5×10^{-4}	21.4 ± 5.55	0	
	1×10^{-4}		0	
	5×10^{-5}	18.2 ± 4.78	0	
	1×10^{-5}		4	
	5×10^{-6}	8.4 ± 2.41	14	
		Experiment B		
Dibutyryl cyclic AMP	0		39	19
	1×10^{-2}*		0	63

* Dibutyryl cyclic AMP did not influence the bioassay for histamine or the level of spontaneous leak, which remained at less than 8 percent.

concentrations of cyclic AMP prevented osmotic lysis of lysosomes (*10*). Although the bidirectional effect of cyclic AMP on the IgE-dependent antigen-induced release of chemical mediators from human lung tissue has been related to the secretory nature of that reaction, the findings that the inhibitory action of cyclic AMP is preceded by several biochemical events in that reaction (*2*) and that the inhibition is manifest against cytolytic reactions as noted herein suggest that an increase in endogenous cyclic AMP affects IgE-dependent antigen-induced and cytolytic mediator release by an action either at different sites or at a common site involving both cellular and granular membranes.

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16 July 1973; 6 November 1973

Parathyroid Hormone Effects in Rats Treated with Diphosphonate

Abstract. *The ability of disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP; 40 milligrams per kilogram of body weight per day) to reduce the hypercalcemic effect of parathyroid hormone in thyroparathyroidectomized rats was confirmed. However, treatment with this large dose of EHDP enhanced the hypophosphatemic effect of a low dose of parathyroid hormone (10 international units per 100 grams of body weight), apparently by promoting the renal excretion of phosphate. The data suggest that EHDP may have a direct effect on the renal action of parathyroid hormone and, in this way, may also affect vitamin D metabolism by the kidney.*

Diphosphonates have been reported to inhibit bone resorption (*1*, *2*) and to prevent soft tissue calcification (*3*). At least part of the skeletal effect has been attributed to the binding of the pyrophosphate analog on the surface of apatite crystals (*4*). In rats, disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP), at a daily dose of 40 mg per kilogram of body weight for 3 days, reduced the hypocalcemic response to calcitonin but did not affect the hypophosphatemic action of the hormone (*5*). A similar reduction in the hypercalcemic response of EHDP-treated rats to parathyroid hormone (PTH) also has been reported (*6*). The studies reported here suggest that the renal response of the rat to PTH is not inhibited by pretreatment with this dose of EHDP, but in fact actually may be enhanced.

Male rats weighing between 140 to 200 g (Zivic-Miller strain) were injected subcutaneously with EHDP, 40 mg kg^{-1} day^{-1} (I. Y. Rosenblum, Procter & Gamble Co.), between 9 and 10 a.m. for five successive days. This dose level may have been toxic since it reduced the gain in weight of these growing rats by 25 percent. On the afternoon of the fifth day, all rats were thyroparathyroidectomized (TPTX), and food and water were continuously supplied. The morning of the sixth day, a single subcutaneous injection of PTH, either 10 or 20 international units (I.U.) per 100 g of body weight (Wilson Laboratories, 250 units per milligram of trichloroacetic acid powder), was administered. Blood samples (from tail vein) were obtained prior to hormone injection and at 1 and 3 hours after injection. In one experiment the rats were bilaterally nephrectomized 2 hours prior to injection of PTH. Plasma calcium (Ca) and inorganic phosphate (P_i) were determined by standard methods (*7*, *8*). Statistical comparisons were made by Student's *t*-test.

In preliminary experiments the following reported effects of EHDP treatment in rats were confirmed: (i) Ca and P_i fluxes into bone were markedly reduced, as were the rates of removal of previously deposited radionuclides; (ii) after 5 days of treatment, the concentration of plasma Ca was slightly elevated, while the concentration of plasma P_i was significantly lowered; and (iii) following TPTX or parathyroidectomy, the rate of fall in plasma Ca and the rate of rise in plasma P_i were significantly reduced by treatment with EHDP. The inhibition of the ability of PTH (10 to 20 I.U. per 100 g of body weight) to raise plasma Ca concentrations in TPTX rats was also confirmed; however, these experiments suggested that the hypophosphatemic action of PTH was enhanced by 5 days of treatment with EHDP, despite the fact that plasma P_i concentrations at the time of PTH injection were between 1.0 and 1.5 mg/100 ml lower than in controls. The experiments described below confirmed this finding.

Using the standardized procedures (18 hours after TPTX; animals either fasted or fed), we made a comparison of the immediate effects of PTH on plasma Ca and P_i in control rats and in those treated with EHDP. The data, summarized in Fig. 1, demonstrate that in EHDP-treated rats the hypercalcemic action of PTH was reduced while its hypophosphatemic effect was augmented. The increased hypophosphatemia in EHDP-treated animals after injection of PTH was more apparent at the lower dose of the hormone and at 1 hour after its injection than at the higher dose or at 3 hours after injection of PTH. A similar sequence of changes was observed in plasma ^{45}Ca and ^{32}P activities when the radionuclides were injected 3 to 6 days prior to PTH administration.

Preliminary data on the effect of EHDP on renal excretion of Ca and P_i have been obtained. Based on the values of both P_i and ^{32}P in urine, the increase in P_i excretion following administration of PTH was greater in EHDP-treated rats than in nontreated animals. Changes in the concentration of Ca in urine followed a different pattern. In EHDP-treated TPTX rats, urinary Ca was increased almost ten-