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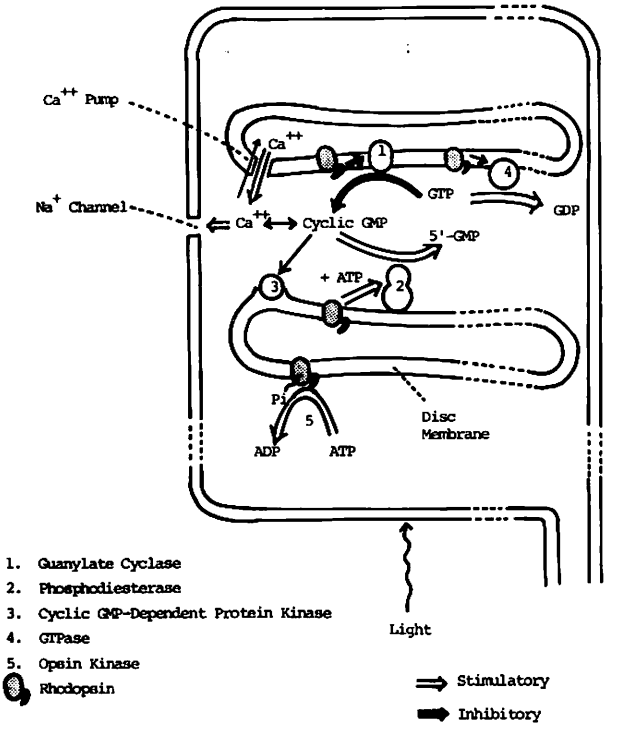
Pharmacology

Cyclic GMP System in Photoreceptor Rod Outer Segment.



DEPARTMENT OF PHARMACOLOGY

GENERAL SUMMARY

Our efforts during the past three years have been devoted to extend the following two works:

1. **Lipid metabolism of lymphoid cells with special reference to their physiological functions.**

In previous studies we reported the occurrence of high levels of cytotoxic free fatty acids in lymphoid cells of normal and tumor-bearing animals (guinea pigs and mice). These results suggest characteristic features of lipid metabolism in lymphoid cells presumably underlying the physiological function of lymphocytes as a surveillance mechanism. Further studies along these lines have revealed the following facts concerning the distribution patterns in the lipid constituents of lymphoid cells.

(1) Adrenergic catecholamines (isoproterenol, epinephrine and norepinephrine) were highly potent in decreasing the triglyceride levels of the splenic lymphoid cells from guinea pigs *in vitro*, while they were quite inert upon the inguinal lymph node cells.

(2) In mice, higher levels of free fatty acids and their esters were demonstrated in the lymphoid cells resistant to cortisone and cyclophosphamide as compared to the cells from normal animals.

(3) A close correlation was found between the levels of free cholesterol and free fatty acids in lymphoid cells from thymus, spleen or lymph node of mice and guinea pigs, suggesting a possible role of cholesterol regulating the fatty acid levels in lymphoid cells.

2. **Properties of guinea-pig serum L-asparaginase.**

In a previous report Ryoyama demonstrated that the stability to heat of the L-asparaginase activity of serum samples of outbred strain (Hartley) guinea-pigs markedly varied with individual sera and further that the heat inactivation of the enzyme activity was totally protected by high concentration of Na^+ or K^+ but not Mg^{++} or Ca^{++}.

In an attempt to clarify the question of the heterogeneity of L-asparaginase in guinea-pig serum, she has studied (a) the effects of urea and 2-mercaptoethanol (protein denaturants) on the serum and liver L-asparaginase, (b) the chemical properties of the purified serum-enzyme preparations obtained from the outbred strain (Hartley) guinea pig and (c) the heat stability of the serum specimens from the four inbred strains of guinea pigs (JY-1, Hartley/F, Strain 2 and Strain 13). The results thus obtained from all these experiments indicate that a possible relation of genetic factor may be important to the thermal stability of L-asparaginase protein in guinea-pig serum, although the intricate mechanism responsible for the enzyme stability is as yet unclear.

ABSTRACT

(43) Lipid metabolism of lymphoid cells with special reference to their physiological functions. (1) The in vitro effect of adrenergic agents and related compounds on triglyceride levels of guinea-pig lymphoid cells.

S. Kigoshi* and R. Ito

There is increasing evidence that several functions of the immune system are intimately associated with the adrenergic receptors in the plasma membrane of lymphocytes[1),2),3)]. In a previous work we reported the characteristic distribution patterns in the lipid constituents of guinea-pig lymphoid cells. This raises the question as to whether the adrenergic drugs, known to stimulate lipolytic activity in adipose tissues, might influence the lipid contents of lymphoid cells. Lymphoid cells were obtained from spleens and inguinal lymph nodes of guinea-pigs of the Hartley strain. Lymphoid cell suspensions (approx. 2.5×10^8 cells) in Krebs-Ringer phosphate buffer, pH 7.4, containing 2% of bovine albumin fraction V were incubated at 37°C for 2 h with or without catecholamines. Triglycerides of the washed cells were extracted and analyzed as reported previously. The values determined from the cells incubated with drugs were evaluated as percentage of the drug-free controls. Of the catecholamines tested, isoproterenol, epinephrine and norepinephrine were capable of producing a similar, but unique for each cell sourse, response to the triglyceride levels of lymphoid cells. When the splenic cells were incubated in 10^{-5}M concentration of the drugs, the triglyceride levels fell to about 25% of the original levels. The response to logarithmically decreasing doses of the 3 drugs decreases in a linear fashion over the range from 10^{-5} to 10^{-10}M. Their 50% decreasing dose was approximately 10^{-8} M. The effect of phenylephrine was considerably less than that observed with the 3 catecholamines, while ephedrine had practically no effect over the range of 10^{-10} -10^{-3}M. In marked contrast, under similar conditions none of the adrenergic agents active upon splenic cells caused any significant change in the triglyceride levels of the lymph node cells. The catecholamine-induced reduction of the lipid contents were unaffected by the presence of the α- and β-adrenergic blockers (phentolamine and propranolol). These results suggest an involvement of lipid metabolism in physiological functioning of lymphocytes through the adrenergic receptor system.

1) Hadden, J. W., Bourne, H. R. and Middleton, E., JR., Cell. Immun., 1, 583 (1970).
2) Strom, T. B., Carpenter, C. B., Garovoy, M. R., Austin, K. F., Merrel, J. P. and Kaliner, M., J. Exp. Med., 138, 381 (1973).
3) Schreiner, G. F. amd Unanue, E. R., J. Immun., 114, 802 (1973).

* Dept. of Pharm., School of Medicine, Kanazawa University.

(44) Lipid metabolism of lymphoid cells with special reference to their physiological functions. (2) High levels of free fatty acids and their esters in lymphoid cells resistant to cortisone or cyclophosphamide.

S. Kigoshi* and M. Akiyama

In mice, systemic administration of corticosteroids results in rapid atrophy of thymus, spleen and lymph nodes, and a pronounced decrease of lymphocytes in these tissues. Cyclophosphamide, and immunosuppressive agents as well as corticosteroid, also has been reported to deplete the lymphocytes present in thymus and other lymphoid tissues of mice. We examined the lipid composition of lymphoid cells from thymus, spleen and mesenteric lymph node of mice treated with hydrocortisone (12.5mg/100g of body weight) or cyclophosphamide (7.5mg/mouse). The quantities of fatty acids, triglycerides and cholesterol esters were about 2–7 times higher in the lymphoid cells from the drug-treated mice than in the cells from normal animals. However, there was only a slight difference in the lipid composition of lymphoid cells between cortisone-treated and cyclophosphamide-treated animals within the respective tissues, except for the triglycerides and cholesterol esters in the thymic lymphoid cells.

These results suggest (a) that the lipid composition of immunocompetent lymphocytes differs significantly from that of immuno-incompetent lymphocytes, and further (b) that the lymphoid cells resistant to immunosuppressive agents markedly differ from the drug-sensitive cells in the significantly high levels of free fatty acids and thier esters within the respective lymphoid tissues of mice.

Table. Lipid composition of lymphoid cells from thymus, spleen and mesenteric lymph node of normal mice and animals treated with either hydrocortisone (HC) or cyclophosphamide (CP).

Tissues	Agents used for treatment	Lipid/Lymphoid cells (mg/10^{10} cells)[a]		
		Fatty acids	Triglycerides	Cholesterol esters
Thymus	Normal	5.9 ± 0.6	6.9 ± 0.8	2.8 ± 0.6
	HC	21.5 ± 1.3	36.9 ± 6.6	20.9 ± 3.1
	CP	15.4 ± 2.1	15.2 ± 1.6	7.8 ± 1.2
Spleen	Normal	6.7 ± 0.3	6.3 ± 1.4	3.6 ± 0.4
	HC	15.0 ± 1.7	37.2 ± 4.2	8.8 ± 0.8
	CP	17.2 ± 1.1	40.8 ± 7.4	10.8 ± 1.0
M.L.N.[b]	Normal	32.9 ± 1.3	13.8 ± 1.2	7.2 ± 0.6
	HC	86.2 ± 3.6	38.5 ± 5.5	12.3 ± 2.1
	CP	61.1 ± 4.5	37.9 ± 4.6	13.4 ± 1.2

All of the mice were fed with diet and given water ad libitum before the experiments. a) Each value is given as mean ± S.E. of 7 experiments. b) Mesenteric lymph node. Lipid contents (mg/10^{10} cells) of mesenteric lymphoid cells from normal mice deprived of diet for 48 hours before the experiments were as follows: fatty acids, 36.8 ± 2.7; triglycerides, 10.2 ± 0.8; cholesterol esters, 6.1 ± 1.0 (number of experiments=7).

(45) Lipid metabolism of lymphoid cells with special reference to their physiological functions. (3) Close correlation between levels of cholesterol and free fatty acids in lymphoid cells.

S. Kigoshi*, M. Akiyama and R. Ito

Recent studies have shown that free cholesterol is involved in the fluidity relating to the rigidity of the cell membrane, and that the cholesterol levels in normal lymphocytes from man and animals differ markedly from that in leukemic cells. Previously, we reported that growth of Ehrlich's ascitic carcinoma in mice results in increase of free cholesterol and free fatty acids in lymphoid cells. The present study demonstrates a close correlation between the levels of free cholesterol and free fatty acids in lymphoid cells from thymus, spleen or lymph nodes of mice (normal and tumor-bearing) and guinea pigs.

The levels of free cholesterol, free fatty acids and phospholipids in lymphoid cells varied greatly with the groups of the animals. Each of the fatty acid values in lymphoid cells was then plotted against the corresponding values of cholesterol within each of the lymphoid tissues. Apparently there was a nearly linear relationship between the levels of cholesterol and fatty acids in lymphoid cells from the tissues of both mice and guinea pigs. In mice the correlation coefficient of fatty acids to cholesterol was 0.94 for thymic cells, 0.99 for splenic cells and 0.95 for cervical lymphoid cells, respectively. On the other hand, the correlation coefficient of phospholipids to cholesterol was 0.70 for thymus, 0.83 for spleen, 0.19 for mesenteric lymph node and 0.24 for cervical lymph node, respectively. In guinea pigs the correlation coefficient of fatty acids to choleaterol was 0.94 for splenic cells and 0.93 for inguinal lymph node cells, respectively, whereas that of phospholipids to cholesterol for splenic and inguinal lymphoid cells was 0.54 and 0.56, respectively.

Recently, it was reported that free cholesterol markedly increases the viscosity of lipid bilayer in the surface membrane of lymphocytes, resulting in the increase of rigidity in the surface membrane[1]. Thus, it appears that, in lymphoid cells from mice and guinea pigs, an increase in the rigidity of surface membrane by free cholesterol is accompanied by an increase of free fatty acids. Turnell et al.[2] have also indicated that, in corticosteroid-sensitive lymphocytes, accumulation of free fatty acids is involved in corticosteroid-induced lymphocytolysis. Free fatty acids from various sources, including lymphocytes, have long been known to be highly cytotoxic to many mammalian cells. Therefore, it is conceivable that free cholesterol regulates the levels of cytotoxic free fatty acids in lymphoid cells by changing the rigidity of the cell membrane.

1) Shinitzky, M. and Inbar, M., J. Molec. Biol., **85**, 603 (1974).
2) Turnell, R, W., Clarke, L. H. and Bourton, A. F., Cancer Res., **33**, 203 (1971).

(46) Effect of strain differences on heat-susceptibility of L-asparaginase in the guinea pig.

C. Ryoyama

Broome reported that antilymphoma and L-asparaginase activities of guinea pig serum were almost entirely lost when treated at 66°C for 30min. In a previous paper, Ryoyama reported that susceptibility of serum L-asparaginase to heat markedly differed among individuals of outbred Hartly guinea pigs tested, and further that the heat-labile enzyme activity was totally protected by high concentration of Na^+ or K^+ but not by Mg^{++} or Ca^{++}. The present study is concerned with the question as to what substance(s) or factor(s) might be related to the thermal stability of serum L-asparaginase.

The effect of urea on the serum L-asparaginase of outbred Hartley guina pigs was examined for comparison between heat-resistant and heat-sensitive types. The heat-resistant serum L-asparaginase was much more stable to urea treatment than the heat-sensitive enzyme, and the urea-inactivation of the enzyme was fairly well protected by Na^+ or K^+. Treatment with 2-mercaptoethanol resulted in slight inactivation of both types of the enzyme, but the inactivation was not influenced by Na^+. Liver L-asparaginase of the guinea pigs, in which the serum enzyme was resistant to heat, was also resistant to heat, and *vice versa*. The heat inactivation of liver enzyme was also protected by Na^+. Similar results were also demonstrated on purified serum L-asparaginase, although the amino acid compositions between the two purified enzyme preparations were slightly different. When the animals having heat-resistant serum L-asparaginase were crossed with each other, serum enzyme of the resultant progenies was also resistant to heat, and *vice versa*.

The enzyme activity in the serum of JY-1 animal was almost entirely heat-stable. The enzyme of Hartley/F guinea pigs was also heat-resistant as well as that in JY-1. In contrast, the enzyme activities of both Strain 2 and Strain 13 were almost lost at 66°C. Serum enzymes of all strains tested were also protected from heat inactivation by Na^+. Further, the enzyme activities in the serum of JY-1 and Hartley/F guinea pigs was much more resistant to urea than that of Strain 2 and Strain 13, corresponding to the relative stability on heat denaturation. In this connection, it is worthy of mention that the leukocyte phenotype of Strain 2 and Strain 13 was B type[1], whereas that of both JY-1 and Hartley/F strains was AD type[2].

All these results stated above seem to indicate that a possible relation of genetic factor may be important to the thermal stability of L-asparaginase protein of guinea pigs, although it is unknown what mechanism relates to the stability of the enzyme molecules.

1) Sato, W. and Weck, A. L., Z. Immunitätsforsch., 144, 49 (1972).
2) Tanaka, Y., Allergy, 25, 10 (1976).

GENERAL SUMMARY (addendum)

I was appointed professor of pharmacology, Cancer Reseach Institute, Kanazawa University on December 1, 1978 after Professor Ryo Ito retired in April, 1978. I want to describe here my scientific background and research project in this laboratory. I was always interested in the intracellular messengers which exist in small amounts in the cells, and are mainly engaged in calcium and cyclic nucleotides in the central nervous system.

After I graduated from Osaka University Medical School in March 1967, I entered the doctral course of Osaka University Medical School with a major in Pharmacology, working with Professor Hiroshi Yoshida, Head of the 1st Department of Pharmacology, Osaka University Medical School. I started to help Dr. Hajime Ishida (Professor, Department of Pharmacology, Tokushima University Dental School) and studied the role of calcium on amylase secretion from the parotid gland, related to excitation- secretion coupling. We reported that increases in intracellular calcium cause the release of amylase from slices of parotid gland (H. Ishida et al., Japan. J. Pharmacol., **21**, 227, 1971), and also that ATP and a low concentration of calcium release amylase from zymogen granules with addition of the cytoplasmic factor (H. Ishida et al., Japan. J. Pharmacol., **21**, 239, 1971).

Cyclic AMP, as well as calcium attracts me because it was proposed that cyclic AMP is an intracellular second messenger for various hormones, and β-adrenergic activity emerges through cyclic AMP synthesis in the cells. I started to work with calcium-activated phoshodiesterase in the central nervous system toward the PhD degree. We found the existence of an inhibitory protein of phosphodiesterase in the brain, which is sensitive to trypsin digestion (N, Miki et al., Biochim. Biophys. Acta, **268**, 166, 1972).

After completing the doctral course, Professor Iwao Yamamoto invited me to work in the Department of Pharamacology, Osaka University Dental School and I became interested in cyclic nucleotides metabolism in the central nervous system. We suggested that accumulation of cyclic AMP in the cerebral slices is produced by decreases in Na^+ gradient across the cell membrane (I. Yamamoto et al., Japan. J, Pharmacol., **28**, 375, 1978). Soon I was fortunate to have a chance to carry out photoreceptor work with Professor Mark W. Bitensky, Department of Pathology, Yale University Medical School for three years. I worked with cyclic GMP metabolism in photoreceptor cells of the frog retina by light. We found that cyclic GMP phosphodiesterase in photoreceptor cells is markedly activated by ATP and a small amout of light (N. Miki et al., Proc. Natl. Acad. Sci., **70**, 3820, 1973). Our interest then focused on the physiological role of cyclic GMP in photoreceptor cells and the molecular mechanism for the activation of cyclic GMP phosphodiesterase by light and ATP. We tried to purify it, and reported that photoreceptor phosphodiesterase is activated through bleached rhodopsin and an extrinsic protein of the disc membrane. (N.

Miki et al., J. Biol. Chem., **250**, 6320, 1975). I also studied, helping professor Mark W. Bitensky, the activation of adenylate cyclase by cholera toxin. We found that adenylate cyclase from the cancer cell membranes is activated by subunit A of cholera toxin in the presence of NAD (M. W. Bitensky et al., Proc. Natl. Acad. Sci., **72**, 2572, 1975).

After coming back to Japan in 1975, I joined Professor Kinya Kuriyama, Head of Department of Pharmacology, Kyoto Prefectural University of Medicine. I was fortunate to be able to continue studies on cyclic GMP metabolism in the central nervous system. We reported that catalase activates guanylate cyclase in the presence of NaN_3 or NH_2OH. We also suggested NO radical may stimulate guanylate cyclase (N. Miki et al., Biochem. Biophys. Res. Comm., **72**, 953, 1976; N. Miki et al., ibid., **75**, 851, 1977). It is now proposed that cyclic GMP is closely related to cell growth, and that some radical reactions produced in the cells induce normal cells to become cancerous or aged cells. I am interested in the physiological role of cyclic GMP, especially its relation to cell growth and differentiation.

Proposed Research

1. First proposed research seeks information on the physiological role of cyclic GMP.

i) We are planning to examine the responsiveness of the cyclic GMP system (guanylate cyclase, phosphodiesterase, cyclic GMP-dependent protein kinase, cyclic GMP contents) to the radical reactions produced by carcinogenic compounds, enzymatic reaction, light, radiation, metal ions, lipids etc. We also introduce inhibitors of chemical carcinogenesis and radical scavengers such as butylated hydroxytoluene, vitamin A, hemoglobin, anticarcinogenic drugs to modify the cyclic GMP system, cell growth and cell differentiation. There is much evidence that cyclic GMP is closely involved in cell growth and differentiation, and also that nitrosamines, which are potent carcinogenic compounds, activate guanylate cyclase. Radical scavengers or inhibitors of chemical carcinegenesis also inhibit NO radical-stimulated guanylate cyclase. We will examine how cyclic GMP is involved in cancer cells and cell differentiation. We will test the possibility that the carcinogenic compounds or inhibitors of chemical carcinogenesis can be screened using this cyclic GMP system.

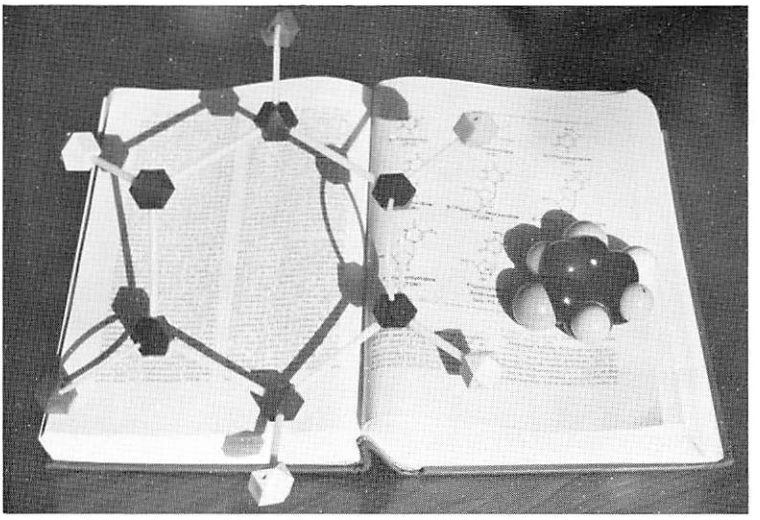
ii) We will continue photoreceptor work because photoreceptor cells in the retina are best for studying the physiological role of cyclic GMP. We will examine whether or not cyclic GMP could be an intracellular messenger of light stimuli in photoreceptor cells. No cyclic GMP-dependent protein kinase activity and no physiological activators of guanylate cyclase have been reported in photoreceptor cells. We believe that the detection of protein kinase activity and guanylate cyclase activators is the clue to solving this research project.

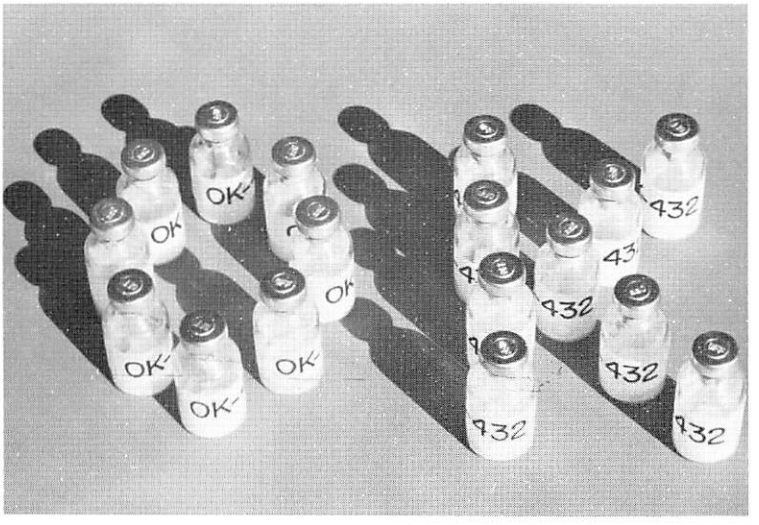
2. The second proposed research seeks the other intracellular messen-

gers or receptors closely associated with cell growth and differentiation, and information about their physiological roles.

We believe some substances or specific receptors appear at a critical period during the cell growth and differentiation. These substances or receptors include cytoplasmic component in egg, nerve growth factor, neurotropic factor and some specific receptors in the cell membrane. They are reported to play an important role in cell growth and differentiation. We will introduce nervous or glial cells, for this project, as well as organs from the central nervous system, and brain tumor cells grown in the culture system (using the tissue culture and eyeball). A microassay system such as radioimmunoassay and enzymatic cycling assay is indispensable for this experiment.

Experimental Therapeutics

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