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INHIBITORY ACTION OF ADENOSINE 3',5'-MONOPHOSPHATE ON PHOSPHATIDYL-INOSITOL TURNOVER: DIFFERENCE IN TISSUE RESPONSE*

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SUMMARY: There appear to be considerable differences among tissues in the inhibitory action of adenosine 3',5'-monophosphate (cyclic AMP) on phosphatidylinositol (PI) turnover induced by various extracellular signals. The present studies were on human peripheral lymphocytes and rat hepatocytes. In the lymphocyte system, cells are activated by phytohemagglutinin that induces PI turnover, and this PI turnover and cellular activation are profoundly blocked by dibutyryl cyclic AMP as well as by prostaglandin E1 which markedly increases cyclic AMP. In contrast, in the hepatocyte system, glycogenolysis is enhanced by α-agonists that induce PI turnover as well as by β-agonists and glucagon that increase cyclic AMP. In these cells the two classes of receptors appear to function independently, and PI turnover is not inhibited by cyclic AMP.

In most tissues there appear to be two major receptor mechanisms for the control of cellular activities by various extracellular signals. One is related to cyclic AMP,1/ and the other seems to be implicated in

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1/ The abbreviations used are: cyclic AMP, adenosine 3',5'-monophosphate; PI, phosphatidylinositol; PHA, phytohemagglutinin; PGE1, prostaglandin E1; DBcAMP, dibutyryl cyclic AMP.

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Ca^{2+} actions (for review, see Ref. 1). In general, the stimulation of the latter class of receptors induces PI turnover. This phospholipid turnover was first recognized by Hokin and Hokin (2) in acetylcholine-sensitive excretory glands and subsequently shown by many investigators in various tissues activated by hormones, neurotransmitters and various other biologically active substances (for review, see Ref. 3). Presumably, this mode of tissue response can be roughly divided into two types: In most systems, such as lymphocytes, leukocytes, platelets and smooth muscle, the receptors that induce PI turnover promote activation of cellular functions, whereas the receptors that produce cyclic AMP usually antagonize such activation, although the mechanism of this antagonism remains unclear. In contrast in the other type of system the two classes of receptors do not appear to interact with each other but function independently. For instance, in hepatic tissue, α- and β-agonists, which induce PI turnover and cyclic AMP accumulation, respectively, enhance glycogenolysis equally (4,5). It has been well established that cyclic AMP plays roles through activation of cyclic AMP-dependent protein kinase. In an analogous manner it is proposed that PI turnover may be coupled to the activation of another species of protein kinase, that is Ca^{2+}-activated, phospholipid-dependent protein kinase recently found in various tissues (6-8). In the present studies using lymphocytes and hepatocytes, we examined whether cyclic AMP interacts with PI turnover. The results showed that cyclic AMP inhibits PI turnover in lymphocytes but not in hepatocytes. Ca^{2+}-activated, phospholipid-dependent protein kinase and cyclic AMP-dependent protein kinase are referred to tentatively as protein kinases C and A, respectively.

EXPERIMENTAL PROCEDURES

Cells———Washed human peripheral lymphocytes were prepared by the method of Böyum (9). The preparation consisted of 90-95% lymphocytes and 5-10% granulocytes and/or monocytes. The lymphocytes were

suspended in Eagle's medium at a concentration of 1 x 10^6 cells/ml, and maintained for 20 hr at 37°C under 5% CO_2 in air. Parenchymal hepatocytes were isolated from adult rats as described earlier (10). The cells (5 x 10^5 cells/ml) were plated in Williams' medium E supplemented with 5% calf serum and 10 μM dexamethasone, and cultured for 7 hr at 37°C under 5% CO_2, 45% O_2 and 50% N_2. These cells accumulated glycogen and maintained various hepatic functions, including responses to hormones such as glucagon, epinephrine and insulin, urea synthesis, albumin synthesis and enzyme induction as described (11).

Materials and Chemicals——The materials and chemicals used for preparation of lymphocytes and hepatocytes were obtained as described earlier (10,12). PHA and PGE1 were products of Difco Laboratories and Ono Pharmaceutical Co., respectively. DBcAMP, glucagon and phenylephrine were purchased from Sigma. Epinephrine and isoproterenol were obtained from Nakarai Chemicals. ^{32}Pi and [^{3}H]thymidine (5 Ci/mmol) were from the Radiochemical Centre. Other chemicals were obtained from commercial sources.

Procedures and Determinations——PI turnover was usually assayed by measuring the incorporation of ^{32}P into PI as described originally by Hokin and Hokin (2) and later established by many investigators (3). The radioactive phospholipids were extracted directly with chloroform/methanol (1:2) by the method of Bligh and Dyer (13), isolated by thin layer chromatography on Silica Gel H (E. Merck) plates, and determined as described earlier (14). DNA synthesis was assayed by measuring the incorporation of [^{3}H]thymidine into acid-precipitable materials as described by Smith et al. (15). Glucose output from hepatocytes was assayed enzymatically with glucose oxidase as described (16). Cyclic AMP was determined by radioimmunoassay by the method of Steiner et al. (17). Other conditions are specified in each experiment. The radioactivity of ^{32}P- and ^{3}H-samples was determined using a Packard Tri-Carb liquid scintillation spectrometer, Model 3320.

RESULTS AND DISCUSSION

Polyclonal plant mitogens such as PHA induce rapid PI turnover in lymphocytes (18,19). The results in Table I confirm with the cells employed in the present studies that the incorporation of ^{32}P into PI was greatly increased by PHA, but that practically no enhancement of labelling was observed with other major phospholipids. The enhancement of labelling of PI was observed immediately after the addition of PHA, and the reaction continued linearly for at least 60 min. The radioactivity recovered in the phosphatidic acid fraction was very small, although the labelling pattern of this phospholipid showed great diversity in different tissues (data not shown). The poor labelling of phosphatidic acid in lymphocytes was presumably due to its rapid

Table I

PHA-induced enhancement of ^{32}P-incorporation into PI in human peripheral lymphocytes

Phospholipid	Without PHA	With PHA
Phosphatidylinositol	690	4,060
Phosphatidic acid	400	500
Phosphatidylserine	1,950	1,730
Phosphatidylethanolamine	5,000	5,700
Phosphatidylcholine	3,400	3,600

Human peripheral lymphocytes were suspended in phosphate-free Krebs-Ringer bicarbonate buffer at pH 7.5 at a concentration of 1 x 10^6 cells/ml. The suspension was incubated with carrier-free ^{32}Pi (30 μCi/ml) for 1 hr at 37°C, and then stimulated by PHA (10 μg/1 x 10^6 cells) for 30 min. Phospholipids were extracted and isolated by thin layer chromatography using a solvent system of chloroform-methanol-acetic acid-water (50:30:8:4). For separating PI and phosphatidylserine an additional solvent system of methyl-acetate-n-propanol-chloroform-methanol-0.25% aqueous KCl (25:25:25:10:9) was employed. The numbers indicate the radioactivity (counts per min) incorporated into each phospholipid.

conversion to PI by way of CDP-diacylglycerol (PI turnover). Later, DNA synthesis was enhanced and the rate of [^3H]thymidine incorporation into the acid-precipitable fraction was maximal 72 hr after PHA challenge. Although the causal relationship between the enhancement of PI turnover and DNA synthesis is not clear, both entities were equally inhibited by DBcAMP as well as by PGE1, which markedly increased cyclic AMP as shown in Table II. Sodium butyrate per se had no effect. The more quantitative results shown in Fig. 1 indicate that both incorporation of ^{32}P into PI and that of [^3H]thymidine into DNA were inhibited progressively by increasing amounts of PGE1, and this inhibition was inversely related to the cyclic AMP level. This inhibitory effect of PGE1 was reproduced by DBcAMP. Agents increasing the cyclic AMP level, such as PGE1 and cholera toxin, are known to prevent PHA-induced alterations of lymphocytes, such as enhancement of Ca^{2+} trans-

Table II

Inhibition by PGE1 and DBcAMP of PHA-induced enhancement of ^{32}P-incorporation into PI and [^{3}H]thymidine-incorporation into DNA in lymphocytes

Addition	^{32}P-Incorporation into PI	[^{3}H]Thymidine-incorporation into DNA	Cyclic AMP formation
	(cpm)	(cpm)	(pmol/1 x 10^6 cells)
None	830	420	0.2
PHA (10 μg/ml)	3,630	1,710	0.2
PGE1 (1 x 10^{-5} M)	820	430	2.2
DBcAMP (1 x 10^{-3} M)	790	510	—
PHA + PGE1	1,450	670	3.0
PHA + DBcAMP	1,250	560	—

^{32}P-Incorporation into PI was assayed as described in the legend to Table I except that PGE1 and DBcAMP were added as indicated during cell activation. For the assay of DNA synthesis, non-radioactive lymphocytes (1 x 10^6 cells/ml) suspended in Eagle's medium were stimulated by PHA for 72 hr in the presence and absence of either PGE1 or DBcAMP as indicated, and then incubated with [^{3}H] thymidine (1 μCi/ml) for 2 hr at 37°C. Cyclic AMP was measured after incubation of the cells for 10 min at 37°C with and without PHA and PGE1 as indicated.

port, glucose transport, sterol and fatty acid syntheses and RNA and DNA syntheses (for review, see Ref. 20). The mechanism of this inhibitory action of cyclic AMP is not clear, cyclic AMP may block PI turnover and thereby counteract the activation of a multifunctional protein kinase that is protein kinase C. Similar inhibition by cyclic AMP of PI turnover was also observed in other cell types which may be activated by various extracellular signals but inhibited by another group of extracellular signals that increase cyclic AMP. For instance, in human platelets stimulated by thrombin and rat polymorphonuclear leukocytes stimulated by a chemoattractant, PI turnover and cellular activation were blocked concurrently by DBcAMP as well as by PGE1 which markedly elevated the cyclic AMP level (data not shown).

The above findings raise the question of whether cyclic AMP inhi-

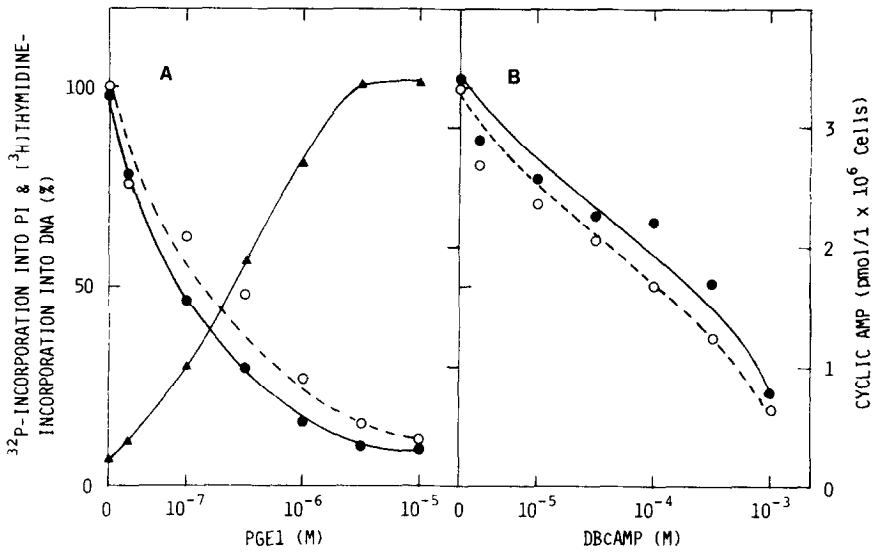

Fig. 1. Effects of various concentrations of PGE1 and DBcAMP on PHA-induced enhancement of ^{32}P-incorporation into PI and [^{3}H]thymidine-incorporation into DNA in lymphocytes. ^{32}P-Incorporation into PI, [^{3}H]thymidine-incorporation into DNA, and cyclic AMP were measured under conditions similar to those described in the legend to Table II except that various concentrations of PGE1 and DBcAMP were added as indicated. A, with PGE1; B, with DBcAMP. (●——●), ^{32}P-incorporation into PI; (o-----o), [^{3}H]thymidine-incorporation into DNA; (▲——▲), cyclic AMP.

bits PI turnover in the other type of system such as liver where the two receptors function independently. In isolated hepatocytes it seems established that glycogenolysis is enhanced by β-agonists and glucagon through a cyclic AMP-dependent pathway. It is also well known that α-agonists, vasopressin and angiotensin II provoke PI turnover as well as glycogenolysis without any detectable increase in the cyclic AMP level and without activation of protein kinase A (3,4). The experiments summarized in Table III showed that PI turnover was enhanced by α-agonist but not by β-agonist. Under these conditions this enhanced PI turnover was not inhibited by β-agonist, glucagon or DBcAMP. Preliminary analysis indicated that insulin did not inhibit PI turnover.

Table III

Effects of cyclic AMP-elevating compounds on α-agonist-induced enhancement of ^{32}P-incorporation into PI in rat hepatocytes

Addition	^{32}P-Incorporation into PI	Glucose output	Cyclic AMP formation
	(cpm)	(μg/hr/mg of protein)	(pmol/mg of protein)
None	5,850	46	2.5
Epinephrine (1×10^{-5} M)	12,260	100	69.9
Phenylephrine (1×10^{-5} M)	10,820	83	8.4
Isoproterenol (1×10^{-5} M)	5,430	102	79.9
Glucagon (1×10^{-7} M)	5,390	95	149
DBcAMP (1×10^{-4} M)	5,380	110	—
Phenylephrine + Isoproterenol	10,180	104	83
Phenylephrine + Glucagon	9,680	105	138
Phenylephrine + DBcAMP	10,940	110	—

Cultured rat hepatocytes (1×10^6 cells/35 mm dish) were incubated with carrier-free ^{32}Pi (50 μCi/ml) for 1 hr at 37°C, and then stimulated for 30 min at 37°C by various compounds as indicated. ^{32}P-Incorporation into PI was measured as described in the legend to Table I. Glucose output was assayed after incubation of the cells for 30 min at 37°C with various compounds as indicated in glucose-free Hanks-Hepes buffer at pH 7.2. Cyclic AMP was measured after incubation of the cells for 10 min at 37°C with various compounds as indicated.

Protein kinase C is widely distributed in mammalian tissues including all the cell types mentioned above (8). A series of studies with human platelets stimulated by thrombin strongly suggested that this protein kinase is activated by diacylglycerol, which is derived by the receptor-linked hydrolysis of PI, and is directly involved in protein phosphorylation during platelet activation (21,22). Although the relationship between PI turnover and the cellular response has not yet been clarified for lymphocytes and hepatocytes, it seems attractive to suggest that in the type of system such as lymphocytes, the receptor-linked activation of protein kinase C is blocked by cyclic AMP, presumably through the action of protein kinase A, whereas

in the type of system such as liver, protein kinases C and A function independently in their specific roles in controlling cellular activities. The inhibition of PI turnover by protein kinase A does not appear to be due simply to the phosphorylation of phospholipase C, and the molecular basis of this difference in tissue response remains unexplored.

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