cyclic amp: the ubiquitous hormone

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It is now being claimed that many hormones act by way of a two-messenger system. The hormones may be regarded as first messengers which travel from their cells of origin to their target tissues to stimulate the formation therein of a second, intracellular messenger. Although one cannot exclude that other second messengers will eventually be discovered, the only one identified so far is cyclic adenosine 3', 5' monophosphate, or cyclic AMP (cAMP, Fig. 1). The function of this cyclic nucleotide as second messenger mediating the effects of a variety of hormones and other biologically active agents has now been definitely proved. Since the recognition of its physiological role, from the discovery that it mediated the hyperglycaemic effect of adrenaline and glucagon by stimulating the conversion of inactive to active glycogen phosphorylase, work in many laboratories has established both the ubiquity of cAMP in living organisms and the large number of its regulatory mechanisms.

ADENYL CYCLASE AND cAMP-DIESTERASE

The level of cAMP inside the cells at any given instant depends upon the activities of at least two enzymes: adenyl cyclase and phosphodiesterase.

Adenyl Cyclase. Adenyl cyclase catalyses the formation of cAMP and pyrophosphate from adenosine triphosphate (ATP) in a reaction which requires Mg++ :

$$ATP \longrightarrow cAMP + PPi$$

It is present in both nucleated and akaryotic cells where it is associated with either the cytoplasmic (e.g. in fat-cells, liver), mitochondrial (e.g. in skeletal muscle) or endoplasmic membrane (e.g. in fat-cells). Its profound metabolic importance resides in the fact that its activity responds to a wide variety of hormones (TABLE I) and other pharmacolgically active compounds.

The mechanism of its hormonal activation is generally assumed to involve an allosteric interaction between the hormone and either the adenyl cyclase or the membrane with which it is associated. The same adenyl cyclase may be stimulated by different effectors; conversely, the adenyl cyclases of individual tissues may vary in their response to the same effectors. Thus there arise problems as to the reasons for the hormonal specificity of adenyl cyclase.

It would appear that the molecular configuration of at least part of the adenyl cyclase system must differ from one tissue to another, or even within the same cells. The existence of distinct adenyl cyclase-receptor complexes in the various parts of the kidney (Marumo and Edelman, 1971) and of two distinct adenyl cyclases in the liver has been claimed. Most evidence supports the hypothesis that stimulation by different effectors is mediated by separate specific receptors at the outer surface of the plasma membrance; some of the receptors are at least partially proteinaceous. Competition between activators of differing efficacy probably also occurs; prostaglandin E_1 (PGE_1), a less potent activator of renal medullary adenyl cyclase than antidiuretic hormone (ADH), decreases ADH-mediated increases in cAMP production by competing with ADH for the receptors which influence adenyl cyclase in rat medulla (Beck et al., 1971). Cyclase stimulation decreases with age.

cAMP-Diesterase. Phosphodiesterase catalyses the hydrolysis of cAMP to 5'-AMP; it is specific for the 3', 5' diester-bond. Its distribution parallels that of adenyl cyclase and may occur, in some cases (e.g. in brain cortex) even in the same cell, in soluble (e.g. in liver) as well as in particulate form. The activity of the enzyme increases with age.

PLASMA cAMP

Under basal conditions, the plasma level of cAMP, whose turnover is known to be rapid, seems to be maintained by mechanisms that involve both uptake and release by tissue, metabolism within tissue, urinary excretion, and possibly other mechanisms (Blonde et al., 1974).

The small intestine is a site of net production of cAMP, and so may be the lungs (Wehmann et al., 1974); the kidneys are responsible for about 5% of the total rate of entry into plasma of cAMP (Blonde et al., 1974). Nevertheless, renal elimination accounts for 30% of the plasma clearance rate of cAMP, 10% as a result of metabolism within the renal tissue and 20% accounted for by urinary excretion; 30-55% of the urinary cAMP is derived from production and release by the renal parenchyma, the rest from glomerular filtration (Blonde et al., 1974). The liver is also one important site of elimination of plasma cAMP; a balance between uptake and secretion by this organ may exist (Blonde et al., 1974). However, plasma cAMP is present in such low concentration relative to intracellular concentrations that it is unlikely to be acting as a hormone by penetrating cell membranes of selected tissues (Blonde et al., 1974).

cAMP AND THE SYNTHESIS OF PROTEINS

Synthesis of a number of enzymes in various organs is stimulated by cAMP which appears to stimulate either the transcription to the specific messenger or its transmission from nucleus to cytoplasm.

Effect of cAMP on the transcription of genes. The mechanism by which cAMP stimulates the synthesis of functional messenger is unknown. The hypothesis that the phosphorylation of histones and other nuclear proteins mediates the differential transcription of genes is supported by the finding that cAMP stimulates the phosphorylation of purified liver histone kinase. Furthermore, cAMP increases the rate of

TABLE I. INTERACTIONS BETWEEN HORMONES AND ADENYL CYCLASES*

Organ producing hormone	Hormone	Adenyl Cyclase of
Adrenal medulla	Adrenaline	Brain Lung Spleen Pineal gland Salivary gland Ocular tissue Heart Aorta Diaphragm Skeletal muscle Liver Erythrocytes Fat cells Oviduct or uterus
	Nor-adrenaline	Fat cells Aorta Brain Pineal gland Hypophysis Salivary gland Oviduct or uterus Ocular tissue Melanocytes
All mammalian tissues	Prosta-glandins	Hypophysis Thyroid Lung Diaphragm Heart Aorta Skeletal muscle Spleen Blood platelets Kidney Adrenal cortex? Corpus luteum
Testis	Testosterone	Testis?
Intestine	Secretin	Fat cells
Pancreas	Glucagon	Heart Liver Blood platelets Fat cells
Placenta	Gonadotropin	Testis
Ovary	Oestrogen	Oviduct and uterus
Kidney	Erythropoietin?	Bone marrow
Neuro-hypophysis	Antidiuretic hormone	Aorta Kidney Urinary bladder
	Oxytocin	Urinary bladder
Hypo-thalamus	Hypothalamic extract	Hypophysis
Para-thyroid	Para-thyroid hormone	Skeletal muscles Bone Kidney
Thyroid	Triiodo-thyronine	Heart Corpus luteum Spermatozoid
	Thyro-calcitonin	Kidney Bone
Adeno-hypophysis	Luteinising hormone	Fat cells Corpus luteum
	Adrenocort-icotropin	Adrenal cortex Fat cells
	Thyroid stimulating hormone	Thyroid Fat cells
Pars intermedia	Melanocyte stimulating hormone	Melanocytes
	Phytohaem-agglutinin	Lymphocytes

*Reproduced and modified from Jost and Rickenberg: Cyclic AMP. Ann. Rev. Biochem. **40:** 741-74, 1971.

RNA synthesis in purified rat liver and the increase is preceded by phosphorylation of non-ohistone proteins and f_1 histones. Moreover various observations suggest that interactions of tissue-specific nuclear protein kinases, substrate and cAMP may be important in the tissue-specific regulations of RNA synthesis and chromatin function (Kish and Kleinsmith, 1974).

Effect of cAMP on the translation of mRNA. Besides the effect on the transcription of certain genes, an effect of cAMP on the translation of a bacterial messenger has also been claimed. The synthesis of tryptophanase is controlled at the level of the polysome. The ribosomal G-factor (a protein which participates in the translation of the nascent polypeptide chain) binds cAMP in the simultaneous presence of guanosine triphosphate (GTP). It has been suggested that cAMP regulates the movement of ribosomes along the messenger RNA (mRNA), so as to slow its hydrolysis by RNase V.

cAMP and inhibition of protein synthesis. Inhibition of protein synthesis by cAMP has been reported to occur in liver and muscle fibers.

EFFECT OF cAMP ON THE ACTIVITY OF ENZYMES

Glycogen phosphorylase. cAMP regulates glycogen metabolism at two sites. At the first it acts as an allosteric cofactor in a reaction in which a protein kinase, phosphorylase b kinase kinase, catalyses the phosphorylation of seryl residues of inactive phosphorylase b kinase converting the enzyme into its active form; ATP serves as phosphate donor in this reaction which is completely dependent on the presence of cAMP. The active phosphorylase b kinase catalyses the activation of glycogen phosphorylase b to phosphorylase a:

2 Phosphorylase b + 4 ATP
(inactive)
| Active Phosphorylase b Kinase
↓
Phosphorylase a + 4 ADP
(active)

Under certain conditions cAMP reduces the activity of phosphorylase a phosphatase which catalyses the inactivating dephosphosphorylation of phosphorylase a to b in the reaction

Phrosphorylase a + 4 H_2O
(active)
| Phosphorylase a phosphatase
↓
2 Phosphorylase b + 4 Pi
(inactive)

Glycogen phosphorylase. cAMP regulates glycogen metabolism at a second site. Here it stimulates the conversion of the active, dephosphorylated I form of glycogen synthetase to the inactive, phosphorylated D form by activating glycogen synthetase kinase which catalyses the reaction

Synthetase I + ATP
(active)
| Active Synthetase Kinase
↓
Synthetase D + ADP
(inactive)

Insulin blocks glucagon-stimulated glucose release from liver mainly by diverting glucose into glycogen (Mayo Johnson et al., 1972) and probably acts at this site by antagonising the cAMP production mediated by glucagon, which in turn acts by activating hepatic adenylate cyclase. (Liljenquist et al., 1974).

Phosphofructokinase. cAMP plays a complex role in activating phosphofructokinase, the enzyme catalysing the rate-limiting step in glycolysis. At least in mammalian heart it exerts two distinct effects. It overcomes the inhibition of phosphofructokinase by ATP at subsaturating concentrations of fructose-6-phosphate in a partially competitive manner, and also appears to participate in the activation of the inactive form of phosphofructokinase, this activation involving subunit aggregation.

Miscellaneous enzymes. cAMP counteracts the effect of ATP on the susceptibility of glyceraldehyde-3-phosphate dehydrogenase to inactivation by chymotrypsin. It stimulates the oxidation of glutamate, alpha-ketoglutarate, and pyruvate by brain homogenates and mitochondria. It also overcomes the inhibition of pyruvate kinase by ATP.

Protein kinases. cAMP stimulates protein kinase activity by dissociating the kinase into an inhibitory and a catalytically active subunit; the inhibitory component binds cAMP. In human erythrocytes both protein kinase components are localised on the inner, cytoplasmic surface of the plasma membrane (Rubin et al., 1973). A heat-stable protein which inhibits activation of muscle kinase by cAMP has been isolated from skeletal muscle; little is known about its mode of action.

cAMP AND LIPOLYSIS

cAMP mediates the effect of a number of lipolytic hormones (Fig. 2) and stimulates lipolysis in white and brown fat cells. Where various lipolytic hormones act on the same tissue (e.g. fat cells), the different hormones either interact with different sites or subunits of the same adenyl cyclase or different hormonal discriminators interact in the membrane with the same adenyl cyclase. Low K+ concentrations inhibit lipolysis, possibly by decreasing the affinity of the enzyme for hormones; it has been shown that K+ stimulates, but is not essential for, the activation of lipolysis in white fat cells.

Lipolysis is also severely inhibited if oxidative phosphorylation is blocked; Fain et al. (1973) found a good correlation between the ability of catecholamines and serotonin to affect cAMP accumulation, lipolysis, and respiration in brown fat cells. Thus stimulation of lipase activity by cAMP requires ATP and therefore may be effected by phosphorylation.

High concentrations of triodothyronine enhance the sensitivity of adipose tissue to the action of lipolytic hormones, whilst hydrocortisone is required for the maximal stimulation of adenyl cyclase by catecholamines and ACTH. Beta-adrenergic blocking agents are able to block the formation of cAMP in response to catecholamines but not to other hormones. The lipolytic and most other effects of catecholamines are decreased by acidosis and increased

by alkalosis. Whereas prostaglandins stimulate the adenyl cyclase of the adrenals and of the corpus luteum, PG_1, PGA and PG_2[a], inhnibit that of fat cells. The lipolytic effect of glucagon may be obscured in vivo by the antilipolytic effect of insulin whose release it tends to stimulate.

cAMP AND STEROIDOGENESIS

Several steroidogenic hormones, including ACTH, luteinising hormone (LH) and prostaglandins (E_2, E_7, F_1[a]), stimulate the adenyl cyclase of their steroidogenic target tissues (e.g., adrenal cortex, testis, and corpus luteum) and the cAMP, in turn, activates different steps of steroidogenesis (Fig. 3). Only a small fraction of the cells' potential to synthesise cAMP need be activated to achieve maximum steroidogenesis (Schulster et al., 1972).

cAMP appears to stimulate three different steps in steroid biosynthesis. The first step, the conversion of cholesterol ester to cholesterol, does not require protein synthesis, but the second, the conversion of cholesterol to pregnenolone, does; Mahaffee et al. (1974), however, suggest that cAMP stimulates steroidogenesis by regulating the mitochondrial precursor pool of cholesterol, rather than through a direct effect on the mitochondrial enzyme system that transforms cholesterol to pregnenolone. Stimulation of the third step, the conversion of 11-deoxycorticosterone to corticosterone, appears to involve an activation of the C-11 beta-hydroxylase. cAMP also inhibits (competitively with NAD) the two enzymes which catalyse the conversion of pregnenolone to progesterone: D^5 - 3beta - hydroxysteroid dehydrogenase and D^5 - 3 - ketosteroid isomerase. A cAMP-binding protein has recently been purified from the adrenals.

Current concepts for the complicated mechanism whereby ACTH stimulates corticosteroidogenesis therefore involve the following aspects: ACTH binding at cell-surface receptors, activation of adenyl cyclase and increased production of intracellular cAMP, activation of protein kinases with increased phosphorylation of ribosomal protein, induction of labile protein(s) implicated in the rate-limiting step of steroidogenesis and intracellular translocation of steroid intermediates that influence cholesterol conversion into pregnenolone (Schulster et al., 1972). Rubin et al. (1972) propose that ACTH activates adenyl cyclase by displacing calcium from some site on or near adenyl cyclase. They also suggest that the translocation of this calcium fraction into the cell interior to some active site — possibly the endoplasmic reticulum of mitochondria — which couples steroid production and release may be responsible for initiating steroid release. Angiotensin interacts synergistically with ACTH in elevating cAMP levels to stimulate steroidogenesis in bovine fasciculata cells, but not in human cells owing probably to the use of safely-low doses. (Peytremann et al., 1973).

cAMP AND CHOLESTEROL AND FATTY ACID SYNTHESIS

Bricker and Levey (1972) suggest that cAMP, probably under the control of some unknown primary hormone, may be involved in regulating acetyl-CoA incorporation into de novo fatty acid and cholesterol, and hence lipid synthesis (Capuzzi et al., 1974), in a specific manner in mammalian liver.

THE PERMISSIVE EFFECT OF GLUCOCORTICOIDS ON THE BIOLOGICAL ACTIVITY OF cAMP

Adrenal glucocorticoids have been implicated in the control of many metabolic processes involving cAMP, such as in glycogen synthesis and degradation, gluconeogenesis, lipolysis, and protein synthesis; they may play a similar role in gastric acid secretion (Domschke et al., 1972). In general they exert their effect at a site beyond that of the synthesis of cAMP, but a glucocorticoid has in fact been found to be required to mediate the stimulation of adenyl cyclase by ACTH (but not by adrenaline, glucagon, or fluoride) in fat-cell membrane.

Moreover, the permissive effect of hydrocortisone on the regulatory activity of cAMP requires the synthesis of protein. However, the possibility that glucocorticoids cause the synthesis of proteins known to interact with cAMP, such as cAMP-stimulated protein kinases, has apparently not been tested (Jost and Rickenberg, 1971).

Fig. 4 presents a model for the interaction between glucocorticoids and cAMP. Evidently the model, particularly as regards the effect of cAMP on transcription, is tentative. It is assumed that glucocorticoids stimulate the synthesis of several proteins, including that of protein phosphokinases, and regulatory elements of membranal adenyl cyclases; that is, the effect of glucocorticoids would be twofold: an increase in the concentration of cAMP and of proteins that interact with cAMP. Cyclic AMP, in both the cytoplasm and the nucleus, enhances the activity of protein kinases and presumably also interacts directly with other proteins. The phosphorylation (or other cAMP-linked alteration) of a nuclear protein (acidic, histone?) may then lead to the depression of specific genes.

cAMP AND THE PERMEABILITY OF MEMBRANES

Water. ADH enhances the permeability of certain epithelial membranes, for example, in the kidney, to water, sodium, and other low molecular weight substances such as urea through the synthesis and accumulation of cAMP. The effects on osmotic flow and permeability to urea may be mediated by a single pool of cAMP. Delorenzo et al. (1973) suggest that the effect of cAMP on sodium and/or water transport in toad-bladder membrane might be mediated through the level of phosphorylation of a specific protein; ADH and cAMP decrease the phosphorylation of this specific protein, partially through the activation, in the presence of cAMP, of a membrane-bound phosphoprotein phosphatase.

Ca^{++} inhibited the basal level of adenyl cyclase activity in golden hamster kidney and ADH did not overcome this inhibition (Marumo

and Edelman, 1971). PGE_1, a less potent activator of adenyl cyclase than ADH, decreases ADH-mediated increases in renal medullary cAMP production by competing with ADH for the receptors which influence adenyl cyclase (Beck et al., 1971). Stoff et al. (1972) claim a permissive effect of aldosterone on the permeability responses to ADH mediated by a steroid-dependent increase in the accumulation of cAMP in the pertinent epithelial cells, probably as a consequence of a diminution in the rate of degradation of the intracellular nucleotide.

Catecholamines which stimulate adrenergic alpha-receptors inhibit the response to ADH in the toad-bladder. Goodman et al. (1972) reported that acute or sustained metabolic acidosis or alkalosis did not affect, in any significant way at least, the level of renal cortical cAMP; this also implies that cAMP does not mediate the effects of acidosis and alkalosis on renal ammoniagenesis (Goodman et al., 1972).

Sodium. ADH and cAMP consistently enhanced the short-circuit current and sodium transport across frog skin. It has been suggested that the increase in the sodium-dependent short-circuit current is related to the reduction in concentration of protein disulphide groups (through the action of cAMP) and possibly to a change in membrane structure, resulting in enhanced permeability to Na^+.

Potassium. Adrenaline, glucagon and cAMP cause an efflux of K^+ from the liver; that in response to glucagon precedes activation of glycogen phosphorylase. A variety of lipolytic agents such as adrenaline, ACTH, and cAMP increase the efflux of K^+ from isolated fat cells.

Calcium. Glucagon and cAMP cause an immediate efflux of Ca^{++}, preceding that of K^+, from liver. Dibutyryl cAMP caused mobilisation of Ca^{++} from bone, an effect blocked by thyrocalcitonin. Glucagon enhances Ca^{++} accumulation by the heart muscle during excitation, an effect mediated at least in part by cAMP. Transport of Ca^{++} in intestinal mucosa is also affected by cAMP and is apparently dependent on the presence of a factor induced by vitamin D. It has been suggested that $Ca++$ transport plays an important role in the regulation of gluconeogenesis and glycolysis. It has been speculated that shifts in the intracellular distribution of Ca^{++} are responsible for many, if not all, of the effects of cAMP.

Amino-acids. cAMP also appears to stimulate the uptake of amino-acids in a variety of tissues. Thus uptake of alpha-aminoisobutyric acid was stimulated in rat livers perfused with glucagon or cAMP. An enhancement of the transport of amino-acids requires a much higher concentration of exogenous cAMP than a stimulation of glycogenolysis.

cAMP AND SECRETION

Insulin release. It would appear that glucagon stimulates insulin release by activating adenyl cyclase; the mechanism by which cAMP might act to stimulate insulin release is unknown. Charles et al. (1973) obtained results suggesting a minor role for cAMP in directly stimulating insulin release but a prominent role in modulating glucose-induced release of insulin; such modulation may be achieved by an effect on the movement of granules in the beta-cell (Montague and Cook, 1971). It seems possible that the mode of action of cAMP in stimulating insulin release may be to promote microtubular function by increasing the phosphorylation of microtubular proteins (Lacy et al., 1968; Montague and Howell, 1972). Both diazoxide and imidazole inhibited insulin release from mammalian pancreas but diazoxide, unlike imidazole, was without significant effect on the activity of the cAMP-diesterase (Sams and Montague, 1972).

Growth hormone secretion. Evidence suggests that cAMP also mediates the release of growth hormone (GH) by an action requiring ionised calcium (Lockhart Ewart and Taylor, 1971; Cooper et al., 1972) and a source of metabolic energy (Lockhart Ewart and Taylor, 1971). The mechanism is independent of pituitary protein synthesis de novo, but the integrity of the glycolytic pathway of glucose metabolism appears to be essential (Lockhart Ewart and Taylor, 1971). A microfilamentous or microtubular protein may be involved (Cooper et al., 1972). It is now suggested that cAMP may be the specific trigger of the migration of storage granules to the cell periphery for discharge. The cyclic nucleotide has been invoked as an activator, together with Ca^{++}, of a contractile microtubular system, as seems to be involved also in the secretion of insulin from the pancreatic cells, of catecholamines from perfused adrenals and of thyroid hormones.

Gastric acid secretion. Results obtained by Bieck et al. (1973) indicate that hydrochloric acid secretion into the gastric juice is mediated by cAMP. Histamine, which stimulates gastric acid secretion, stimulates gastric mucosal adenylate cyclase via interaction with H_2 receptor without influencing cAMP breakdown (Dousa and Code, 1974). Domschke et al. (1972) claim that glucocorticoids may play a permissive role in gastric acid secretion via a permissive action on net cAMP production.

Pancreatic exocrine secretion. Case and Scratcherd (1972) claim that the action of secretin, but not that of pancreozymin, may be mediated through cAMP.

Salivary secretion. Amylase secretion from the parotid gland in response to catecholamines, which seems to be mediated via adrenergic beta-receptors, is mediated by increases in level of cAMP.

cAMP AND SYNAPTIC AND NEUROMUSCULAR TRANSMISSION

Synaptic transmission. According to a hypothesis advanced by Johnson et al. (1972) and supported by the results of other workers (McAfee and Greengard, 1972), cAMP, generated in postsynaptic neurons in response to transmitter released during synaptic transmission, activates a specific protein constituent of the postsynaptic plasma membrane; this process results in an alteration of the permeability of the membrane to inorganic ions, thereby bringing

about the hyperpolarisation of the membrane. It has also been proposed that the first step in neurosecretion is a cAMP-mediated phosphorylation of a neurotubular protein (Cooper et al., 1972).

Neuromuscular transmission. Facilitation of neuromuscular transmission by adrenaline seems to involve cAMP (Robison et al, 1968). Cyclic AMP levels increase in skeletal muscle in response to both adrenaline and high K^+, whose effects seems to be additive. The implication in the present case is that cAMP may play a role in the release of acetylcholine from nerve endings.

cAMP AND CARDIAC FUNCTION

Evidence supports the hypothesis that the positive inotropic effect of the catecholamines, of glucagon and possibly of histamine on cardiac muscle is mediated by cAMP. This positive inotropic response can be dissociated from the activation of cardiac phosphorylase. In fact whilst it has not been possible to dissociate the increased level of cAMP from the inotropic response, data suggest that the cAMP-dependent activity of phosphorylase b kinase might have to exceed a certain threshold before it becomes capable of catalysing the phosphorylase b-to-a reaction under the conditions prevailing within the myofibrils. Glucagon enhances Ca^{++} accumulation by heart muscle during excitation and the positive inotropic effect of the hormone, as also that of adrenaline, may result from an increase in the sarcotubular Ca^{++} pool of the heart muscle; the effect is mediated at least in part via cAMP. It has also been suggested that many cardioactive drugs affect the intracellular distribution and cellular concentration of Ca^{++} via cAMP.

cAMP AND ADRENERGIC ALPHA- AND BETA-RECEPTORS

Evidence indicates that in general adrenergic beta-receptor effects are mediated by an increase and alpha-receptor effects by a decrease in the intracellular level of cAMP. A puzzling fact, however, is that in intestinal smooth muscle a releasing action of the catecholamines has been produced both by stimulating adrenergic alpha- and beta-receptors; with adrenaline and noradrenaline a mixed alpha- and beta-receptor response has been obtained. The relaxation mediated by beta-receptors has been found to be preceded by and correlated with an increase in the cAMP content, whereas alpha-receptor relaxation after some delay is associated with a reduction of the cAMP content. Adrenergic beta-receptor stimulation is also combined with an increase of the phosphorylase a activity and a transient reduction of the concentration of the ATP and creatine phosphate contents of the muscle. Andersson and Nilsson (1972) reported that their findings indicated a relationship between relaxation of intestinal smooth muscle and an increased content of cAMP; they also found that cAMP stimulated the binding of Ca^{++} in a microsomal fraction of smooth muscle under utilisation of ATP and claimed that the relaxing action was probably dependent on a decrease of the free myoplasmic Ca^{++} concentration.

cAMP AND PHOTORECEPTOR PHYSIOLOGY

Studies by Miki et al. (1973) showed that illumination markedly diminishes the concentration of cyclic nucleotides in suspensions of photoreceptor membranes, but the locus of regulation is cyclic nucleotide phosphodiesterase (light-stimulated) and not adenylate cyclase. The process of activation of phosphodiesterase by light is in two steps, a light-dependent step followed by an ATP-dependent step. Illumination (in the absence of ATP) produces a trypsin-resistant, heat-labile, macromolocular stimulator. In the presence of ATP the stimulator increases the activity of photoreceptor phosphodiesterase. The light-produced stimulator appears unique to the photoreceptor membranes and does not activate phosphodiesterase in other tissues.

MODULATION OF INFLAMMATION AND IMMUNITY BY cAMP

From results of various in vitro studies, Bourne et al. (1974) have constructed a hypothesis by which certain hormones and mediators of inflammation act in vivo on neutrophils, mast cells and basophils to limit the intensity and extent of inflammatory, allergic or anaphylactic reactions, and to modify the function of immunologically-competent cells (lymphocytes) at several stages, from the recognition of an antigenic signal, through its amplification by clonal proliferation, to the expression of an immune response by differentiated T and B effector lymphocytes; this regulatory action is mediated by a general inhibitory action of cAMP on immunologic and inflammatory functions of leucocytes. This hypothesis suggests that certain vasoactive hormones, mediators of inflammation, and cAMP serve to protect the host from the dangerous consequences of an unregulated immune response. The hypothesis is based on the supposition that some or all of the in vitro observations that led to it reflect events that occur in vivo.

Such in vitro observations have yielded two main results. First, they have demonstrated receptors for a strikingly consistent array of vasodilating hormones — histamine, beta-adrenergic catecholamines, prostaglandins of the E series — in basophils, mast cells, neutrophils and T and B lymphocytes. It is beginning to appear that in the case of lymphocytes such specific and distinct receptors are not present in random fashion on all lymphocytes, but may in fact develop concomitantly with the commitment of a clone of immunologically-competent cells to expression of either cell-mediated or humoral immune responses.

Secondly, it has been shown that in contrast to cAMP's stimulation of secretion in other cells, the nucleotide consistently inhibits secretory events in leucocytes thought to be necessary for expression of immune responses. It inhibits the antigen-induced release of histamine from basophils, and of histamine and SRS-A ('slow reacting substance of anaphylaxis') from the primate lung (Orange and Austen, 1971). It also inhibits the cytolysis by sensitised T-lymphocytes of cells bearing the appropriate alloantigen, and prevents the secretion of interferon and possibly

even that a migratory inhibitory factor (MIF), thought to be responsible for the characteristic collection of mononuclear cells in delayed hypersensitivity skin reactions. The nucleotide also appears to inhibit either the production or the secretion of antibody by B lymphocytes following antigenic stimulation. Moreover, there is inconclusive evidence that neutrophil cAMP inhibits the phagocytosis-induced release of beta-glucuronidase and other lysosomal hydrolases by human neutrophils.

It is also possible that cAMP plays a role in the communication between different cell types (between T and B lymphocytes or between subpopulations of T cells) apparently occurring in the early stages of an immune response and determining either its amplification or its suppression; such a possibility has not yet, however, been carefully considered. Studies also suggest that cAMP may inhibit amplification of an immune response, whilst guanosine 3', 5'-monophosphate (cGMP) may enhance it (Watson et al., 1973). It is being suggested that cGMP and cAMP may act in a "push-pull" fashion to regulate functions of lymphocytes, neutrophils, and even mast cells: wherever cAMP appears to inhibit a reaction, cGMP may enhance it; similarly, wherever sympathetic neurohormones (catecholamines) appear to act through cAMP, the parasympathetic neurotransmitter acetylcholine (or its congeners) may act through cGMP. Cyclic GMP should therefore perhaps be included in the hypothesis of Bourne et al.

An essential feature of the above hypothesis concerns the nature of the hormones themselves, which originate either from neuroendocrine cells (the catecholamines) or from inflammatory reactions. In the first case the catecholamines provide a neuroendocrine mechanism allowing non-antigenic environmental influences to modify inflammatory or immune responses. In the latter case, the inflammatory mediators would be produced by reaction to tissue injury or by immunologic reactions per se, such as anaphylaxis; thus, the intensity or extent of a response to a specific stimulus could regulate subsequent responses to continued or repeated stimuli — the essential feature of feed-back circuits or servo mechanisms.

cAMP AND GROWTH AND MORPHOGENESIS

Cell growth and contact inhibition. Evidence points to an inhibition of mitotic activity by increased levels of cAMP (Marks and Grimm, 1971; Bronstad et al., 1971). Frank (1972) found that accumulation of cAMP within rat embryonic cells caused a strong decrease of thymidine incorporation and of cell proliferation; the cells were stopped in G1 phase of the cell cycle probably due to the interaction of cAMP with one or more metabolic pathways (Frank, 1972). Furthermore, many cells divide in logarithmic phase until they become confluent with other cells, at which time growth ceases or continues at a diminished rate (Burk, 1968; Otten et al., 1972). cAMP concentration remains low during logarithmic growth, increases when the cells approach confluency, and rises to even higher levels several days after the cells have stopped dividing; the contact-inhibited (Stoker and Rubin, 1967) cells are stopped in G1 phase. Washing away of the accumulated cAMP by serum reactivates 'resting' cells (Frank, 1972; Otten et al., 1972), probably through the activation of a cAMP-phosphodiesterase (Frank, 1972). Evidence therefore points to a common regulator mechanism for both contact inhibition and growth regulation of cell-cultures by serum (Frank, 1972); increases in cAMP levels could therefore be due to depletion of some serum factor (Otten et al., 1972).

Cell differentiation. Luin et al. (1973) observed that the morphology and histotypic pattern of some mammalian cells in culture can be changed from a less differentiated to a more differentiated state by agents raising intracellular cAMP. Tash and Mann (1973) found an immediate decrease in the level of cAMP in spermatozoa in response to agents that either depress the motility or shorten the life-span of spermatozoa; thus senescence of spermatozoa seems to be closely related to a decrease in cAMP levels.

cAMP and Turnover Tissue. Konnek et al. (1973) observed that cAMP incorporated in the growth media of either carcinoma or sarcoma cells in continuous culture causes not only a marked growth inhibition but also a dramatic alteration in cell morphology towards that of the normal differentiated cell. Apparently related to the morphology and growth inhibition are changes in the cell surface. These cell surface modifications induced by cAMP and apparently shared by normal cells require de novo mRNA synthesis which is associated with an increase in DNA-directed polymerase II activity. The facts that cell surface modifications induced by cAMP depend on mRNA transcription, are reversible and short-lived, and are closely related to the cell cycle of the normal cell, suggest that the universal property of cell division may be regulated by the relative transcriptional activity of one or more genes continuously producing mRNA, which govern properties of the cell surface.

Evidence moreover suggests that the properties of chalone, a cell-specific substance contained in a number of tissues and able to depress the mitotic activity of the corresponding cells in these tissues, may be related to the adenylate cyclase/cAMP system (Cooper and Smith, 1973). Both regulate cell kinetics by an inhibitory reaction; both are deficient in the tumour or transformed cell; both can restore controlled growth and contact inhibition to transformed cells; both can be influenced by the same classes of drug and naturally occurring molecules; and both chalone and adenylate cyclase are cell specific (Cooper and Smith, 1973).

Secondary Sexual characteristics. Singhal et al. (1971) claim that cAMP may be involved in triggering the known metabolic actions of androgens on secondary sexual tissue of the rat.

CONCLUSION

Research work is presently aimed toward hypotheses designed to provide a unitary molecular basis for the multiple effects of cAMP.

Various functions of adenyl cyclase have been discussed above. It has been pointed out that Ca++, rather than cAMP, may be the ultimate effector of certain regulatory sequences; Ca++ may be released from membrane ATP-Ca++ complexes by the action of adenyl cyclase, with formation of cAMP. The activation of various protein kinases by cAMP has also been referred to. Jost and Rickenburg (1971) propose that cAMP acts by affecting the interaction of the subunits of certain proteins, and that the multiple effects of cAMP may find their explanation on the basis of the function of the altered proteins; it also follows that cAMP may exert its effect at any metabolic level, provided that a protein (regulatory protein in the nucleus, enzyme, membranal protein, etc.) participates in the reaction.

The role of cAMP is primarily one of modulation. It appears certain that in many cases cAMP interacts with a number of proteins within the same cell; it may be summarised that proteins differ in their affinities for the cyclic nucleotide. Clearly such a hierarchy of affinities would provide a regulatory system of great subtlety. A hormone control (e.g. by glucocorticoids) of the synthesis of proteins that interact with cAMP would provide an additional dimension of both tissue specificity and flexibility.

Although the precise mode of action of cAMP at the molecular level is still within the realm of speculation, one major physiological function of the nucleotide appears to be the coordination of the mobilisation of potential reserves of carbon and energy when readily available sources, such as glucose, become limiting. This regulatory role of cAMP is exemplified by its stimulation of glycogenolysis, lipolysis, and the conversion of amino acids to their keto derivatives in the mammal and by its reversal of catabolite repression in bacteria.

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