Progress report

The role of cyclic adenosine-3', 5'-monophosphate (AMP) in gastrointestinal secretion

During investigations into the hyperglycaemic actions of adrenalin and glucagon, Rall, Sutherland, and Berthet\(^1\), isolated a new compound capable of stimulating glycogenolysis. Subsequent analysis revealed this to be adenosine 3', 5'-monophosphate, or cyclic AMP. Adrenalin and glucagon increase the rate of formation of cyclic AMP, which in turn promotes the formation of active phosphorylase \(a\) from inactive phosphorylase \(b\) and hence stimulates glycogenolysis\(^2\).

Since this discovery, many hormones have been shown to exert their influence on their target tissue by increasing, or decreasing, the steady state level of cyclic AMP in the cell\(^3\). The concept has thus been developed that these hormones act by way of a two-messenger system\(^4\). The hormone, acting as the first messenger, is released from the cell of origin and travels in the blood stream to the target tissues, where it leads to the formation of a second (intracellular) messenger. The only second messenger so far detected is cyclic AMP. The level of cyclic AMP in the cell is controlled by at least two enzymes: adenylyl cyclase, which catalyses its formation from ATP, and phosphodiesterase, which catalyses its hydrolysis to 5'-AMP. Theoretically a hormone could act by influencing either of these enzymes. In practice, it is the activity of the adenylyl cyclase which appears normally to be affected. This is not surprising since adenylyl cyclase is a component of cell membranes and certain intracellular membranous structures, whereas phosphodiesterase is more diffusely distributed within the cell. Phosphodiesterase is competitively inhibited by the methyl xanthines, namely, theophylline, theobromine, and caffeine. Both adenylyl cyclase and phosphodiesterase are present in most cells of higher organisms, one exception being the non-nucleated mammalian erythrocyte\(^4, 5\).

Evidence for the participation of cyclic AMP in hormone action has usually been first obtained on broken cell preparations of the respective target tissues. Adenylyl cyclase activity is assayed by measuring the formation of cyclic AMP from exogenous ATP both with and without the appropriate hormone\(^6, 7\). The level of cyclic AMP in intact cells can also be measured, and the response to hormone stimulation observed. Clearly, if a change in concentration of cyclic AMP is the primary event, it must occur before, or at least should not follow, the simultaneously monitored physiological response.

Using physiological techniques, evidence may also be obtained by testing two further criteria: can the actions of the hormone be mimicked by the addition of exogenous cyclic AMP or one of its derivatives? Can the hormone also be mimicked, or at least be potentiated, by the methyl xanthines (the drugs which inhibit phosphodiesterase activity, thereby preventing the hydrolysis of cyclic AMP and allowing it to accumulate in the cell)?
Using these techniques, cyclic AMP has been implicated in the action of a great number of hormones. However, observations on the mode of action of gastrointestinal hormones have somewhat lagged behind.

Enhancement of ion transport by cyclic AMP was first demonstrated by Orloff and Handler. The marked similarity of effects of cyclic AMP, theophylline, and vasopressin on the osmotic flow of water across isolated toad bladder was, in their opinion, consistent with the view that vasopressin exerts its effect in toad bladder (and by analogy, in kidney) by stimulating the production and accumulation of cyclic AMP in the receptor tissue.

It has long been known that methyl xanthines can both initiate gastric secretion and potentiate histamine-stimulated gastric secretion. With the elucidation of the hypothesis of a second messenger these observations acquired added significance. Interest in cyclic AMP as an intermediate in the gastric secretory mechanism grew after the observation by Alonso and Harris that methyl xanthines stimulated acid secretion and respiration by frog gastric mucosa in vitro. Stimulation was greater than that achieved with histamine. The only structural feature common to both histamine and the methyl xanthines is the imidazole ring. Since imidazole itself inhibits acid secretion by a mechanism not involving competitive inhibition with histamine, and in view of the structural dissimilarities between histamine and the methyl xanthines, it is likely that they act at different sites. Harris and Alonso have also demonstrated that gastric acid secretion and respiration are stimulated in like manner by gastrin and by cyclic AMP. The stimulatory action of dibutyril cyclic AMP (a synthetic derivative which permeates cells more easily) and of theophylline on gastric acid secretion has also been demonstrated in vivo by a technique involving perfusion of the rat gastric mucosal surface with solutions containing these agents. There is, therefore, some evidence linking the actions of histamine and gastrin to cyclic AMP, and it seems quite possible that the second messenger hypothesis may apply in the case of gastric acid secretion.

These observations have led us to test the actions of cyclic AMP and the methyl xanthines on pancreatic secretion, using the isolated, saline-perfused preparation of the cat’s pancreas. There is usually no basal flow of juice in this preparation. However, adding dibutyril cyclic AMP to the perfusion fluid causes a small flow of juice when secretin is not stimulated. This response to cyclic AMP, and also the response to a submaximal dose of secretin, can be markedly potentiated by the simultaneous addition of theophylline to the perfusate. At high concentration theophylline alone is also capable of causing a small stimulation. Thus, secretin too may act via cyclic AMP.

It has recently been shown that gastrointestinal hormones may also influence the transport of water and electrolytes across the small intestine. It appears that gastrin may inhibit intestinal transport. The observation that both cyclic AMP and theophylline increase the secretion of chloride ions and inhibit absorption of sodium ions by the isolated rabbit ileal mucosa is therefore noteworthy.

There have been a number of observations implicating cyclic AMP in the mechanism of enzyme secretion by alimentary glands. Schramm and his colleagues have established that adrenalin is a potent inducer of enzyme secretion in rat parotid slices. Since the work of Sutherland and others indicates that cyclic AMP is a primary intracellular intermediate in the action
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of adrenalin in several tissues\(^3\), Schramm tested the effect of cyclic AMP in his preparation. Both mono- and dibutyryl cyclic AMP induced rapid amylase secretion; theophylline and caffeine were also active\(^{21, 22}\). Employing a similar technique, Kulka and Sternlicht have obtained similar results using whole mouse pancreas\(^{23}\). Amylase secretion was stimulated by pancreozymin, peptavlon, and carbamyl choline; theophylline, cyclic AMP, and its monoo- and dibutyl derivatives were also potent stimulators of secretion. Kulka and Sternlicht also noticed that 3'-AMP (but not 5'-AMP) inhibited secretion induced by cyclic AMP in a competitive manner. Since 3'-AMP also inhibited secretion stimulated by the hormones, the authors suggest that they also induce secretion via the formation of cyclic AMP. Similar observations have been made by Ridderstrap\(^{24}\) using an intact preparation of the isolated rabbit pancreas, previously described by Rothman and Brooks\(^{25}\). Since, however, secretory rate was not recorded, the increased output of enzymes following the addition of cyclic AMP or theophylline to the bathing fluid may not represent a specific effect on the release of enzymes; it may be secondary to the increased rate of secretion which occurs in response to these agents in the isolated cat pancreas\(^{15}\). We have so far been unable to mimic the action of pancreozymin using cyclic AMP and theophylline in the isolated cat pancreas\(^{15}\). (To our knowledge, there have not yet been any reports linking cyclic AMP with pepsin secretion by the stomach.) Thus there remains some doubt as to whether cyclic AMP acts as the intracellular activator of enzyme secretion.

From the above rather incomplete evidence, it is possible to suggest that cyclic AMP may act as intracellular mediator of some, or all, of the hormones acting on the gastrointestinal tract. The list of hormones acting via cyclic AMP thus grows steadily. How can this single agent, of such ubiquitous distribution, be responsible for the multiplicity of physiological responses characteristic of the many target tissues? Presumably the response must depend on the intracellular environment and the enzymatic profile of the particular tissue involved. Hormone specificity must be maintained by the presence of individual hormone receptors in each cell, or possibly tissue-specific adenyl cyclases.

Knowledge of the biochemical events initiated by cyclic AMP which ultimately result in a secretion of electrolytes and enzymes is still fragmentary. In many tissues cyclic AMP enhances phosphorylase activity, and it has often been supposed that the increased glycogenolysis which follows is responsible for the physiological effect of cyclic AMP in that tissue. Thus in liver, the effect of cyclic AMP on phosphorylase has been shown to be responsible for the observed release of glucose\(^3\). However, there are other examples where phosphorylase can be activated by a mechanism not involving cyclic AMP, or where the increased phosphorylase activity does not accompany the physiological effects of administering cyclic AMP\(^3\). Furthermore, in some tissues the consequences of administering cyclic AMP cannot be explained by an increase in glycolysis, as for example in fat cells, where a lipolytic enzyme system is activated\(^{26}\).

Is the role of cyclic AMP in electrolyte secretion principally to trigger the chemical reactions responsible for supplying energy for the transport processes, or does it affect the transport mechanism directly, with a secondary increase in metabolic rate? Initially Orloff and Handler\(^8\) showed that phosphorylase was unimportant in the regulation of glycogenolysis in the toad bladder.
More recently they have reported that cyclic AMP does enhance glycogenolysis in this tissue and have concluded that vasopressin increases phosphorylase, phosphofructokinase, and pyruvate kinase activity in the toad bladder and that this increase is secondary to the stimulation of sodium transport.

To separate the effects of cyclic AMP on cell metabolism and on the transporting system of the isolated gastric mucosa, Harris and his colleagues have utilized impermeant anions in the bathing solution. Their observations suggest that the primary effect of cyclic AMP is either to influence intermediary metabolism (resulting in an increased secretion) or to affect intermediary metabolism and ion transport separately. Though the effect of cyclic AMP on mucosal phosphorylase is small, there is evidence that some part of glycogenolysis is utilized specifically for gastric acid secretion. Though the small increase in phosphorylase activity does seem to be important, the increase in glycogen utilization as a result of cyclic AMP accounts for only half of the increased oxygen consumption by the mucosa. Harris therefore suggests either that cyclic AMP stimulates lipolysis and glycolysis separately or the increased glycogenolysis and lipolysis are secondary to direct and indirect effects of cyclic AMP on the oxidative phase of metabolism and the secretion process.

Clearly a great deal more information is required before the role of cyclic AMP in transport processes is fully established. Information as to its mode of action in enzyme secretion is virtually non-existent. As in isolated fat cells, it may be that cyclic AMP activates a phospholipase in exocrine glands, resulting in a breakdown of the plasma membrane at the point of fusion with the zymogen granule, thus allowing enzymes to escape. An interesting question in this respect is whether the adenyl cyclase is located in the cell membrane or in the zymogen granules themselves.

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