MECHANISMS OF HORMONE ACTION

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Circulating hormones play an important role in regulating the flux through metabolic pathways. In general, hormones regulate either the activity of key enzymes of metabolism or the activity of protein transport systems such as those found in the plasma membrane and in the inner mitochondrial membrane. Little is known about the mechanism of action of hormones on transport systems, so the discussion in this article will be confined to the mechanism of action of hormones on key enzymes of metabolism.

Hormones regulate the activity of key enzymes of metabolism in two ways. Many hormones whose effects on metabolism develop relatively rapidly (for example glucagon or adrenaline) regulate the activities of enzymes via covalent modifications such as the phosphorylation or dephosphorylation of specific serine residues of the enzyme protein. Such alterations in the phosphorylation state of the enzyme are brought about by changes in the activities of either protein kinases which catalyse phosphorylation reactions or phosphoprotein phosphatases which catalyse dephosphorylation reactions. Phosphorylation or dephosphorylation change the kinetic properties of enzymes in one of two ways: either the maximal catalytic activity of the enzyme changes or the affinity of the enzyme for its substrates, activators or inhibitors changes. As a result, the flux through the enzyme-catalysed reaction changes in response to the hormone. It is likely that other covalent modifications such as the adenylation and deadenylation of specific tyrosine residues also occur in mammalian cells, but so far this covalent modification has been studied only with bacterial enzymes.

Other hormones whose effects on metabolism develop relatively slowly (such as steroid hormones or thyroid hormones) regulate the activities of key enzymes of metabolism via changes in the concentration of enzyme protein. Such changes in enzyme concentration are brought about by alterations in either the rate of enzyme synthesis or the rate of enzyme degradation. Changes in the rate of enzyme synthesis could, in principle, be caused either by changes in the rate of mRNA synthesis, that is transcription control, or by changes in the rate of mRNA utilization for protein synthesis, that is translation control. Changes in the rate of an enzyme's degradation may involve alterations in the activity of a specific protease or changes in the susceptibility of the enzyme to proteolysis. All these processes are potential sites of action of hormones.

There are two general mechanisms of hormone action. Some hormones such as glucagon bind to protein receptors which are located on the surface of the target cell membrane. Binding of these hormones to their cell surface receptors causes a change in the concentration of a specific intracellular molecule known as a second messenger which mediates the effects of the hormone on the metabolism of the target cell. Cyclic AMP has been identified as the second messenger for glucagon action on the liver and adrenaline action on muscle. By contrast, the protein receptors for steroid hormones such as glucocorticoids are located in the cytoplasm of the target cell and hence a mechanism must exist to enable the cell to take up the hormone. Binding of steroid hormones to their cytoplasmic receptors is followed by translocation of the complex to the nucleus, which is the site of action of these hormones.

In this article the role of cyclic AMP as the second messenger for glucagon action on the liver and adrenaline action on muscle is described first. Some recent developments in our understanding of the mechanism of action of adrenaline on liver metabolism are then discussed. Next some ideas concerning the mechanism of action of insulin are discussed in relation to the effect of this hormone on the rate of glycogen synthesis in muscle. Finally, the mechanism of action of hormones such

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as glucocorticoids which regulate the concentration of enzymes such as phosphoenolpyruvate carboxykinase and tyrosine aminotransferase is described.

Cyclic AMP and the mechanisms of action of glucagon on liver metabolism and adrenaline on muscle and adipose tissue metabolism

Glucagon regulates the flux through a number of metabolic pathways in the liver. Thus, glucagon stimulates the rates of glycogen breakdown, gluconeogenesis and ketogenesis and inhibits the rates of glycogen synthesis and fatty acid synthesis in this tissue. It seems that many, if not all, of these effects are mediated exclusively by cyclic AMP-dependent phosphorylation mechanisms.

Circulating glucagon binds to a protein receptor on the surface of the liver cell membrane. The resultant hormone–receptor complex then interacts with the membrane-bound enzyme adenylcyclase and activates it. This enzyme converts cytoplasmic ATP into cyclic AMP which is the second messenger for glucagon action. As a result, the binding of glucagon to its receptor increases the cytoplasmic concentration of cyclic AMP.

However, the intracellular concentration of cyclic AMP is not determined solely by the activity of adenyl cyclase. The membrane-bound enzyme phosphodiesterase converts cyclic AMP into AMP and hence, when active, this enzyme will tend to decrease the cytoplasmic cyclic AMP concentration. One factor which increases the activity of phosphodiesterase is insulin. As a result, insulin can decrease cyclic AMP concentrations in the liver previously increased by glucagon though it should be stressed that insulin has no effect on basal cyclic AMP concentrations. This effect of insulin explains in part its ability to antagonize the effects of glucagon on liver metabolism.

An increase in the cytoplasmic cyclic AMP concentration in the liver causes the activation of the enzyme cyclic AMP-dependent protein kinase. This enzyme is a tetramer consisting of two regulatory subunits and two catalytic subunits. When the regulatory subunits are bound to the catalytic subunits the enzyme is inactive. Cyclic AMP binds to the regulatory subunits and causes dissociation of the enzyme to form free catalytic subunits which are catalytically active. Hence, in the presence of cyclic AMP, the catalytic subunit of cyclic AMP-dependent protein kinase can phosphorylate those key enzymes of metabolism which

are its substrates using the terminal phosphate group of ATP. Enzymes which are substrates for cyclic AMP-dependent protein kinase are characterized by the possession of a short amino acid sequence which is recognized by the kinase. Other factors may regulate the activity of cyclic AMP-dependent protein kinase, since an inhibitor protein for the kinase has been isolated from liver. Factors which regulate the ability of this protein to inhibit the kinase remain to be identified.

Enzymes which are substrates for cyclic AMP-dependent protein kinase in the liver are constantly being identified. Two key enzymes of glycogen metabolism are phosphorylated by the kinase, namely glycogen synthase, which is inhibited, and phosphorylase b kinase, which is activated, thus explaining the ability of glucagon to inhibit glycogen synthesis and stimulate glycogen degradation. Pyruvate kinase and phosphofructokinase are phosphorylated and inhibited while fructose-1, 6-diphosphatase is phosphorylated and activated. As a result glucagon stimulates the rate of gluconeogenesis in the liver. Two key enzymes of fatty acid synthesis, namely ATP citrate lyase and acetyl-CoA carboxylase, are phosphorylated and inhibited and as a result glucagon inhibits the flux through this pathway. Since there are many other substrates for cyclic AMP-dependent protein kinase in the liver, mechanisms must exist to determine which of these enzymes are preferentially phosphorylated in different physiological conditions. Such mechanisms are currently under investigation.

Adrenaline stimulates the rate of glycogen degradation and inhibits the rate of glycogen synthesis in muscle via cyclic AMP-dependent phosphorylation mechanisms. This contrasts with the mechanism of action of adrenaline on glycogen metabolism in the liver (see next section). In muscle, glycogen synthase and phosphorylase b kinase are again substrates for cyclic AMP-dependent protein kinase, thus explaining the effect of adrenaline on glycogen metabolism in this tissue. In adipose tissue the enzyme triglyceride lipase is phosphorylated by the kinase and activated, thus explaining the ability of adrenaline to stimulate the rate of lipolysis in this tissue.

Further details of cyclic AMP-dependent phosphorylation mechanisms and their relationship to the mechanism of action of adrenaline and glucagon on metabolism can be found in Nimmo and Cohen (1977).

Catecholamines, vasopressin and angiotensin II and Ca^{2+} *movements*

The catecholamines adrenaline and nor-adrenaline stimulate the rate of glycogen breakdown in muscle and liver by different mechanisms. In muscle, adrenaline stimulates the activity of adenyl cyclase, increases the concentration of cyclic AMP and activates cyclic AMP-dependent protein kinase. This initiates the series of events described in the previous section, which results in the inhibition of glycogen synthesis and the stimulation of glycogen breakdown in muscle. Furthermore, it has been shown that the effect of adrenaline on glycogen metabolism in muscle is mediated exclusively by this cyclic AMP-dependent mechanism (Dietz et al., 1980). This mechanism for adrenaline action is referred to as the β-adrenergic effect and is initiated by the binding of adrenaline to β receptors located on the surface of the muscle cell membrane.

It has been shown that adrenaline stimulates the rate of glycogen breakdown in the liver by a cyclic AMP-independent mechanism, since compounds such as propranolol, which bind to and block the β receptors, do not interfere with this effect (Sherline, Lynch and Glinsmann, 1972). Adrenaline binds to a second class of receptors, located on the surface of the liver cell membrane, known as α receptors. It has recently become apparent that α-adrenergic effects on the liver are mediated by a Ca^{2+}-dependent mechanism and that other hormones such as vasopressin and angiotensin II also stimulate glycogen breakdown in this tissue by a Ca^{2+}-dependent mechanism.

Adrenaline increases the cytoplasmic Ca^{2+} concentration in the liver cell. Possible sources of Ca^{2+} include the extracellular fluid or intracellular organelles such as the mitochondria or the endoplasmic reticulum. It has been shown that adrenaline stimulates the efflux of Ca^{2+} from intracellular sources and the efflux of Ca^{2+} from the liver cell (Blackmore et al., 1978). Furthermore, recent results suggest that the mitochondria are the most important intracellular source of Ca^{2+} in liver cells, although a role for the endoplasmic reticulum has not been ruled out (Blackmore, Dehaye and Exton, 1979). Hence binding of adrenaline to α receptors on the surface of the liver cell membrane must in some way stimulate the efflux of Ca^{2+} from the mitochondria (see below).

Treatment of liver cells with adrenaline stimulates the phosphorylation of a number of enzymes including glycogen phosphorylase, glycogen synthase and pyruvate kinase (Garrison et al., 1979). This finding explains the ability of adrenaline to stimulate glycogen breakdown, inhibit glycogen synthesis and stimulate the rate of gluconeogenesis in the liver. Since adrenaline acts via a Ca^{2+}-dependent, cyclic AMP-independent mechanism, then a Ca^{2+}-dependent protein kinase must be responsible for these phosphorylations rather than cyclic AMP-dependent protein kinase. Phosphorylase 6 kinase is the best studied example of a Ca^{2+}-dependent protein kinase. However, this enzyme has yet to be purified to homogeneity from liver (Vandenheede, De Wulf and Merlevede, 1979) and as a result no direct evidence exists for the phosphorylation of glycogen phosphorylase, glycogen synthase or pyruvate kinase by phosphorylase b kinase from liver. By contrast, phosphorylase b kinase from skeletal muscle has been purified to homogeneity and its structure determined. It is known that this enzyme contains a δ subunit capable of binding Ca^{2+}. Binding of Ca^{2+} to this regulatory subunit activates phosphorylase b kinase (Shenolikar et al., 1979). The δ subunit is identical to the protein calmodulin, the presence of which confers Ca^{2+} sensitivity on a number of biochemical processes. It is likely that phosphorylase b kinase from liver also contains calmodulin and hence that this enzyme is the Ca^{2+}-dependent protein kinase which mediates α-adrenergic effects on glycogen metabolism and gluconeogenesis in this tissue.

The primary response of the liver cell to the binding of adrenaline to the α receptors remains to be established. Nevertheless, it has been shown that α-adrenergic stimulation of the liver is associated with the breakdown of the phospholipid phosphatidyl inositol (see Michell, Kirk and Billah, 1979). It is likely that the site of phosphatidyl inositol breakdown in the liver cell is the plasma membrane, whereas resynthesis of this phospholipid is associated with the endoplasmic reticulum. The products of phosphatidyl inositol breakdown remain to be precisely characterized, but one of these products is myoinositol 1,2 cyclic phosphate. It has been suggested that this compound is the true second messenger for α-adrenergic effects on the liver, although evidence for this proposal has yet to be provided. However, it has been shown that the increase in the Ca^{2+} concentration in the cytoplasm is secondary to the breakdown of phosphatidyl inositol and that the

two events are in some way coupled, although the link between them has yet to be identified. Hence, if myoinositol 1,2 cyclic phosphate is the true second messenger for α-adrenergic effects, then this molecule must in some way stimulate efflux of Ca^{2+} from the mitochondria. It is apparent from the above discussion that more work is needed before the precise mechanism of α-adrenergic effects on liver metabolism is established.

More details of the mechanism of α-adrenergic effects on liver metabolism are given by Exton (1980).

The mechanism of insulin action

Insulin is often spoken of as the major anabolic hormone of the body because it stimulates the synthesis of glycogen, lipids and protein in a number of tissues. Despite a considerable amount of research into the problem, the mechanism of action of insulin on metabolism remains to be established. Nevertheless, a number of mechanisms have been suggested to account for the actions of insulin on metabolism, but none of these are very satisfactory. For a discussion of the various mechanisms suggested, the reader is referred to a recent review by Czech (1977). It is not intended to repeat the information in that review here, but rather to discuss the mechanism of action of insulin in relation to one of the better understood examples of this hormone's effects on metabolism.

Insulin stimulates the rate of glycogen synthesis in skeletal muscle. The site of action of insulin is the enzyme glycogen synthase, which exists in two forms in skeletal muscle, namely a phosphorylated form which is less catalytically active and a dephosphorylated form which is more catalytically active. It has been shown that administration of insulin in vivo stimulates the activity of glycogen synthase and that this effect can be explained by dephosphorylation of the enzyme (Roach, Rosell-Perez and Larner, 1977). This finding suggests that insulin either stimulates the activity of a phosphoprotein phosphatase or inhibits the activity of a protein kinase. Available evidence favours the former mechanism.

The identity of the phosphoprotein phosphatase which dephosphorylates glycogen synthase has been established and its regulatory properties studied. A protein has been isolated from skeletal muscle which is phosphorylated by cyclic AMP-dependent protein kinase in response to the administration of adrenaline in vivo (Foulkes and Cohen, 1979). When phosphorylated, this protein inhibits the activity of the phosphatase and is therefore referred to as an inhibitor protein. In addition it has been shown that administration of insulin in vivo causes the dephosphorylation of the protein (Foulkes, Jefferson and Cohen, 1980). As a result, the protein no longer inhibits the phosphatase, which is therefore able to dephosphorylate glycogen synthase and hence activates this enzyme. It is likely that, in vivo this protein is a regulatory subunit of the phosphoprotein phosphatase which has become detached from the phosphatase during its purification from muscle.

Since insulin stimulates the dephosphorylation of the inhibitor protein, a second phosphoprotein phosphatase may be involved in mediating the effects of insulin on glycogen synthase in skeletal muscle. The identity of this second phosphoprotein phosphatase and the nature of the factors which regulate its activity remain to be established. However, it is tempting to speculate that insulin stimulates the activity of this second phosphoprotein phosphatase by changing the intracellular concentration of a small molecule which is the second messenger for insulin action.

Possible mechanisms of action of insulin on glycogen synthesis in skeletal muscle have recently been reviewed by Cohen, Nimmo and Proud (1978).

The regulation of enzyme concentration by hormones

In the liver the amino acid tyrosine is degraded via a specific metabolic pathway to give acetoacetate and fumarate. Since fumarate can be converted into glucose via the pathway of gluconeogenesis, then tyrosine is both a glucogenic and a ketogenic amino acid. The first step in the pathway of tyrosine degradation in the liver is catalysed by the enzyme tyrosine aminotransferase. It is likely that the activity of this enzyme controls the flux through the pathway as a whole. The hormonal regulation of tyrosine aminotransferase concentration has been studied intensively.

Glucagon, insulin and glucocorticoids all increase the concentration of liver tyrosine aminotransferase as measured immunochemically. However, it appears that these hormones may act by different mechanisms. The induction of tyrosine aminotransferase activity by the glucocorticoid dexamethasone is characterized by a 1–2 h lag period and is accompanied by an increase in the rate of synthesis of mRNA specific for the enzyme

and in the rate of synthesis of enzyme protein (Olson, Thompson and Granner, 1980). The ability of glucagon to induce tyrosine aminotransferase activity may be dependent upon the simultaneous availability of glucocorticoids. This is the so-called permissive effect of glucocorticoids. It is thought that glucocorticoids act first to increase the concentration of mRNA specific for tyrosine aminotransferase and that the subsequent utilization of this mRNA is controlled by a cyclic AMP- and hence glucagon-sensitive mechanism (Ernest, Chen and Feigelson, 1977). By contrast, it has recently been shown that insulin selectively inhibits the rate of tyrosine aminotransferase degradation in the liver (Spencer et al., 1978).

As previously discussed, the enzyme phosphoenolpyruvate carboxykinase catalyses one of the key reactions of the gluconeogenic pathway. The concentration of this enzyme as measured by immunochemical techniques is regulated by hormones such as glucagon, insulin and glucocorticoids. Thus the glucocorticoid dexamethasone increases the rate of synthesis of phosphoenolpyruvate carboxykinase. By using inhibitors of RNA and protein synthesis, it has been established that glucagon increases the rate of enzyme protein synthesis via changes in the cyclic AMP concentration at a post-transcriptional site. Insulin prevents the increased rate of enzyme protein synthesis in response to glucagon or glucocorticoids (see Gunn et al., 1975, for details). Hence insulin has opposite effects on the rates of synthesis of phosphoenol pyruvate carboxykinase and tyrosine aminotransferase.

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