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COMMENTARY

MECHANISMS INVOLVED IN EFFECTS OF CATECHOLAMINES ON LIVER CARBOHYDRATE METABOLISM

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The second messenger hypothesis of hormone action, which proposes that adenosine 3′:5′-monophosphate (cAMP) acts as the intracellular mediator for a large number of hormones, arose in part from the classic studies of Sutherland and coworkers on the mechanisms of action of glucagon and epinephrine on hepatic glycogenolysis [1]. An updated version of the hypothesis as it applies to hepatic glycogenolysis is depicted in Fig. 1. It illustrates that activation of separate glucagon and β-adrenergic receptors in the plasma membrane of the liver parenchymal cell leads to activation of adenylate cyclase [2]. This enzyme converts ATP to cAMP, leading to intracellular accumulation of cAMP [3]. This nucleotide, in turn, activates an enzyme, cAMP-dependent protein kinase, which phosphorylates certain enzymes with ATP as the phosphoryl donor [4]. The protein kinase is composed of regulatory (R) subunits and catalytic (C) subunits, and the mechanism by which cAMP activates it is as follows [5]. In the absence of stimuli, the enzyme exists predominantly in the holo form (RC) which is inactive because the R subunits exert an inhibitory effect on the C subunits. When the intracellular concentration of cAMP rises due to hormonal stimulation, the nucleotide binds to the R subunits causing them to dissociate from the C subunits. The resulting increase in free, active C subunits is responsible for the increased activity of the kinase. Activated cAMP-dependent protein kinase phosphorylates several key enzymes in the liver cell resulting in alterations in their activity. As shown in Fig. 1, it phosphorylates and activates phosphorylase kinase [6]. This, in turn, phosphorylates phosphorylase *b* converting it to phosphorylase *a*, the more active form of the enzyme. Since phosphorylase catalyzes the rate-limiting reaction of glycogen breakdown, its activation leads to an increase in glycogenolysis and glucose release. Another enzyme acted on by cAMP-dependent protein kinase is glycogen synthase, which catalyzes the rate-limiting reaction of glycogen synthesis [6]. Phosphorylation of this enzyme results in its inactivation and hence inhibition of glycogen formation.

Recently, it has been found that L-type pyruvate

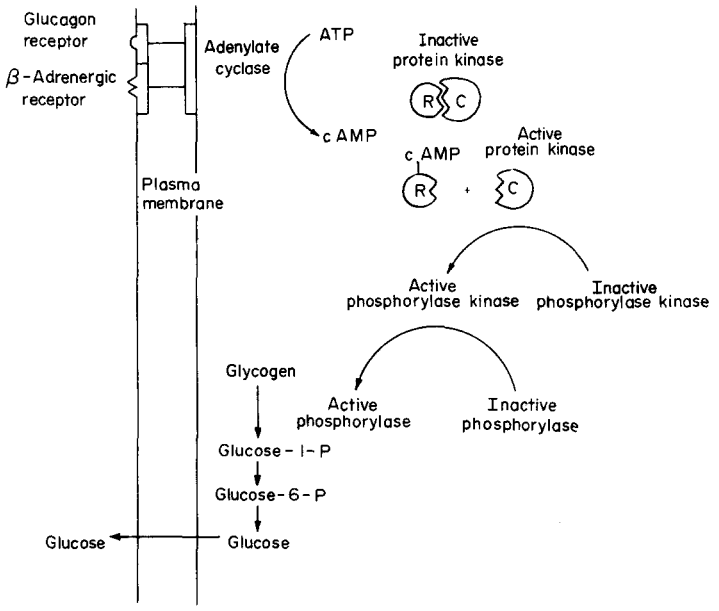

Fig. 1. Updated second messenger hypothesis of Sutherland for hormone stimulation of liver glycogen breakdown.

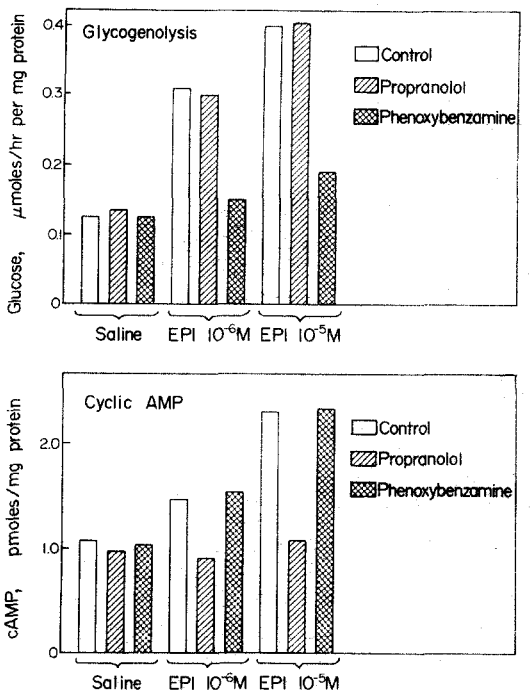

Fig. 2. Effects of adrenergic antagonists on glycogenolysis and cAMP levels in isolated rat hepatocytes incubated with epinephrine.

kinase from liver is also a substrate of cAMP-dependent protein kinase [7]. Phosphorylation of this enzyme causes inactivation, and this appears to be a major component of the stimulatory action of glucagon and epinephrine on hepatic gluconeogenesis [8].

There is much evidence that cAMP is the major, probably only, mediator of the effects of glucagon on the liver [1, 3, 4]. In the case of epinephrine and norepinephrine, however, it is clear that in some species there is another mechanism by which these agents affect the liver [9–11]. This is illustrated by data from experiments with isolated rat liver parenchymal cells (Fig. 2). As expected, epinephrine increased cAMP levels in these cells and, in accord with observations in many tissues, the effect was mediated by β-adrenergic receptors since it was blocked by the β-antagonist propranolol and unaffected by the α-blocker phenoxybenzamine (Fig. 2, lower panel). However, contrary to expectations from the second messenger hypothesis, the glycogenolytic action of epinephrine was not diminished by propranolol, but was decreased by phenoxybenzamine (Fig. 2, upper panel). Similar findings were obtained with norepinephrine and with other α- and β-blockers [10]. Thus, it was concluded that the effects of catecholamines on glycogen breakdown in this system were mediated principally by the α-adrenergic receptor and were less affected by the β-adrenergic receptor-cAMP system [10].

The existence of an α-receptor-mediated, non-cAMP-dependent mechanism(s) for catecholamine action in rat liver is now supported by results from many laboratories [9–17]. It has been shown that α-receptors are important not only in the actions of catecholamines on glycogenolysis [9, 10], but also on gluconeogenesis [10–12] and pyruvate kinase [13, 14], glycogen synthase [10], the stimulation of K^+ and other ion fluxes [15], mitochondrial pyruvate carboxylation [14], and amino acid transport [16, 17]. It is noteworthy that in rat liver the α-adrenergic system causes many of the changes produced by glucagon and β-adrenergic agonists, i.e. the cAMP-independent α-system and the cAMP-dependent β-system frequently provoke the same end responses. This is one of the reasons why investigators have found it difficult to classify the rat "hepatic adrenergic receptor" as α or β [18–21].

It should be emphasized that activation of β-receptors in rat liver does elicit metabolic responses [22], but these are of smaller magnitude than those resulting from α-receptor activation and appear to require higher concentrations of agonist. With epinephrine, a mixed α- and β-agonist, activation of α-receptors alone is generally capable of eliciting maximal metabolic responses; thus, addition of a β-blocker is usually without effect. The small magnitude of the β-adrenergic responses in rat liver is due to the transient, small accumulation of cAMP and not to any impairment in the coupling of cAMP to the physiological responses [22].

Although there is much evidence that α-receptors are important in the hepatic actions of catecholamines in the rat, the situation is less clear in other species. In mouse, cat, rabbit and guinea pig, it has been shown that α-receptors are involved in catecholamine actions on hyperglycemia or hepatic glycogenolysis [9, 10, 18–21, 23–27], but their importance relative to β-receptors is uncertain. It is generally considered that β-receptors mediate the effects of catecholamines on dog liver [19, 20, 28], but the role of α-receptors has not been examined in sufficient detail to justify this view. In man, the question has been explored by comparing the effects of different adrenergic agonists and antagonists on hyperglycemia *in vivo*, and the data indicate a role for α-receptors [18–21]. However, many factors besides direct effects on hepatic glycogen metabolism are probably involved in the blood glucose response to catecholamines, e.g. glucagon release, insulin suppression, lactate gluconeogenesis, and changes in peripheral glucose uptake, so that the interpretation of data is not clear-cut. In short, much more work needs to be done to establish the relative importance of α- and β-receptors in the hepatic effects of epinephrine and norepinephrine in various species.

Although it is clear that α-adrenergic actions in the liver do not involve a rise in cAMP [10–12, 14, 29, 30] or activation of cAMP-dependent protein kinase [29–31], their mechanisms remain uncertain. Despite initial suggestions that guanosine 3′:5′-monophosphate might be involved, there is now much evidence against this possibility.* On the other hand, many investigations point to a probable role for Ca^{2+} ions in α-adrenergic activation of hepatic glycogenolysis. The evidence comprises the following observations: (a) inhibition of hepatic α-adrenergic responses by removal of tissue and medium Ca^{2+} [1, 31–34], (b) α-adrenergic stimulation of ^{45}Ca fluxes and calcium release in liver [31, 32, 34–37], (c) mimicry of α-adrenergically induced glycogenolysis by the divalent cation ionophore A23187 in a calcium-dependent manner [31, 32, 36, 38], and (d) demonstration of the calcium

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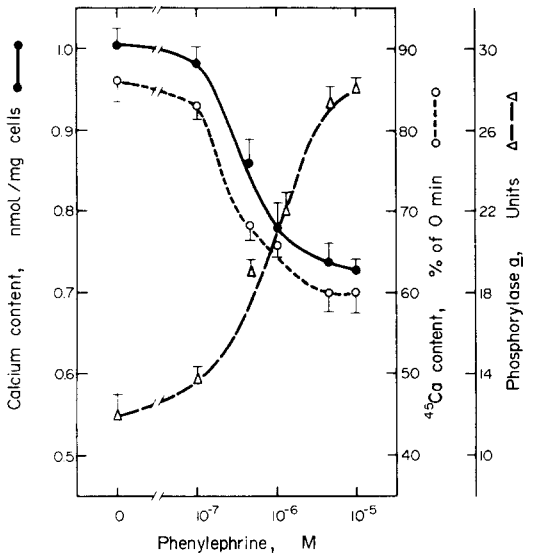

Fig. 3. Dose–response curves for the effects of phenylephrine on phosphorylase *a* levels, calcium content and ^{45}Ca content of rat hepatocytes previously incubated with ^{45}Ca. For details, see Ref. 36. Reproduced by permission of *J. biol. Chem.*

sensitivity of a key enzyme in the phosphorylase activation cascade in liver, namely phosphorylase kinase [39, 40]. Another means of showing the involvement of calcium in α-adrenergic responses, namely inhibition of these responses by calcium antagonists such as verapamil, SKF-525A and La^{3+}, has not proved satisfactory in liver because of the side effects of these agents [41].

Figure 3 from Ref. 36 shows some of the evidence supporting a role for Ca^{2+} ions in α-adrenergic activation of hepatic glycogenolysis. It shows the correlation between the effects of increasing concentrations of the specific α-agonist phenylephrine on phosphorylase activation and the release of Ca^{2+} in isolated rat hepatocytes. A similar correlation is seen with epinephrine, but not with the β-agonist isoproterenol [36]

A general scheme summarizing current information

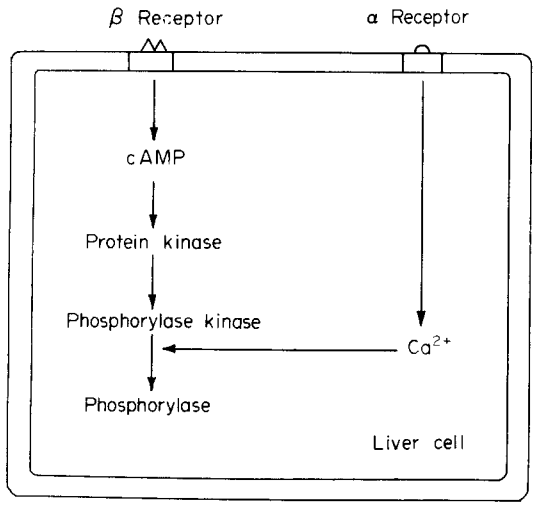

Fig. 4. Dual mechanism for epinephrine stimulation of liver glycogenolysis.

on the mechanisms of α- and β-adrenergic activation of hepatic glycogenolysis is shown in Fig. 4. The β-adrenergic-cAMP mechanism has already been dealt with in detail. The α-adrenergic mechanism is seen to bypass cAMP and cAMP-dependent protein kinase, but to intersect the β-mechanism at phosphorylase kinase via the stimulation of this enzyme by a rise in cytosolic Ca^{2+}.

Many elements of the scheme depicted in Fig. 4 remain uncertain. Liver phosphorylase kinase has not been fully characterized, but it is assumed to have properties similar to those of muscle phosphorylase kinase. Although there is much evidence that it is stimulated by micromolar concentrations of Ca^{2+} [39, 40, 42], there has been no clear demonstration that it is activated or phosphorylated by cAMP-dependent protein kinase. It is conceivable that α-adrenergic activation of phosphorylase could also involve inhibition of phosphorylase phosphatase, the enzyme converting phosphorylase *a* to *b*. However, unpublished experiments in the author's laboratory have shown no effects of α-adrenergic agonists or cytosolic concentrations of Ca^{2+} on this enzyme. The presence of epinephrine binding sites with the characteristics of physiological α-receptors in rat liver plasma membranes has been demonstrated [43], but their possible presence in other structures has not been totally excluded.

There is much evidence that α-adrenergic stimulation mobilizes Ca^{2+} from intracellular stores [34–36], and it is probable that this is the major means by which cytosolic Ca^{2+} is increased. Although influx of extracellular Ca^{2+} appears to occur during α-adrenergic stimulation in other tissues, this is not required for α-adrenergic responses in rat liver [35, 36]. The intracellular site(s) from which Ca^{2+} is mobilized is unknown, but candidates are mitochondria, endoplasmic reticulum and the inner surface of the plasma membrane. Since α-adrenergic stimuli can promote the release of 20–30 per cent of total liver calcium [36], a major calcium storage site must be involved.

The mechanism(s) by which activation of the α-adrenergic receptor leads to mobilization of liver calcium is unknown. It is not known whether another intracellular messenger is involved. Michell [44, 45] and Jones and Michell [46] have proposed that phosphatidylinositol breakdown may be a primary response to stimulation of α-adrenergic or muscarinic cholinergic receptors in various tissues. It remains to be shown whether this is responsible for calcium mobilization or influx, or is a separate effect of α-adrenergic stimulation.

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