5.2.5 Controlling cyclic AMPâ€”Adenylate cyclase and cyclic nucleotide phosphodiesterase

Glycogenolysis and gluconeogenesis in the liver are not only repressed by the hormone insulin, which is released during times of excess nutrient availability, but are also activated by the hormone glucagon, which is released during fasting or starvation. Glucagon acts via a G protein-coupled receptor linked to adenylate cyclase to increase cyclic AMP production (Fig. 5, top right). Miller et al. (2013) found that treatment of primary mouse hepatocytes with phenformin or metformin for 24 h inhibited glucagon-induced cyclic AMP production, with half-maximal effects at around 10 and 200 Î¼M, respectively. Metformin treatment at 250 or 500 Î¼M for 18 h also increased cellular AMP:ATP and ADP:ATP ratios, as would be expected if the drug inhibited Complex I (Section 5.1.1). To explain the decrease in cyclic AMP induced by biguanides, Miller et al. (2013) recalled much earlier findings [e.g., (Fain et al., 1972)] that adenosine and related compounds inhibited adenylate cyclase, and found that AMP inhibited adenylate cyclase in membranes from mouse hepatocytes, although this did require rather high AMP concentrations (300 Î¼M or more). To study these effects in vivo, fed mice were treated with metformin (500 mg/kg) by oral gavage and injected intraperitoneally with glucagon 1 h later. In these experiments, metformin reduced the glucagon-induced enhancement of blood glucose levels and cyclic AMP production, while at the same time increasing cellular AMP levels; similar results were obtained in chow-fed or high fat-fed mice that were fasted overnight to trigger release of endogenous glucagon (Miller et al., 2013).

A different mechanism by which AMPK may depress glucagon-stimulated cyclic AMP production was reported subsequently (Johanns et al., 2016). Cyclic AMP is broken down to AMP by cyclic nucleotide phosphodiesterases. Activation of AMPK in mouse hepatocytes using the compound 991, a potent activator that binds to the ADaM site (see Section 5.2.1) was shown to cause a decrease in glucagon-stimulated cyclic AMP production. The increase in cyclic AMP induced by glucagon was transient, and 991 seemed to affect the declining phase rather than the initial increase, suggesting that it was activating phosphodiesterases rather than inhibiting adenylate cyclases. Indeed, an increase in phosphodiesterase activity that survived cell lysis (suggesting that it was due to a covalent modification) could be measured in cells treated with increasing concentrations of 991, correlating with AMPK activation. Treatment of the cells with metformin (for 24 h) or phenformin (for 2 h) at concentrations of 100 Î¼M or above also caused stable activation of both phosphodiesterase activity and AMPK. The predominant phosphodiesterase isoform in mouse hepatocytes appears to be PDE4B, and AMPK phosphorylated pure recombinant PDE4B in cell-free assays at three sites that were identified by mass spectrometry, accompanied by a twofold increase in maximal phosphodiesterase activity at saturating cyclic AMP concentrations. Mutation of just one of these sites (Ser304) to a non-phosphorylatable alanine residue abolished activation, confirming that phosphorylation of Ser304 was required for AMPK to activate PDE4B. Finally, a phosphospecific antibody recognizing Ser304 on PDE4B was developed, and this revealed that incubation of mouse hepatocytes with either 991 (1â€“10 Î¼M) or phenformin (50â€“500 Î¼M) for 1 h led to phosphorylation at that site. These

data provide rather convincing evidence that activation of AMPK by biguanides in the liver leads to phosphorylation and activation of PDE4B, which reduces cyclic AMP levels.

Thus, the studies from Miller et al. (2013) and Johanns et al. (2016) are in agreement that biguanides can reduce levels of cyclic AMP produced in response to glucagon treatment of hepatocytes, but disagree about the mechanism. How can the two studies be reconciled? There is no obvious reason why the two proposed mechanisms need be mutually exclusive, since biguanides could simultaneously inhibit adenylate cyclase as well as activating cyclic nucleotide phosphodiesterases. Having said that, there do seem to be some clear discrepancies between the experimental results of the two studies. For example, Miller et al. (2013) reported that the reduction in glucagon-stimulated cyclic AMP production induced by phenformin treatment of mouse hepatocytes at 100 Î¼M and above was unaffected by a double knockout of both AMPK catalytic subunit isoforms, and that inhibition by phenformin was still observed in the presence of Ro-20-1724, a specific inhibitor of the PDE4 family of phosphodiesterases. By contrast, Johanns et al. (2016) reported that the effect of the AMPK activator 991 to reduce glucagon-induced cyclic AMP accumulation was abolished by a double knockout of the AMPK catalytic subunits, while the effect was also blocked by rolipram, another specific PDE4 inhibitor. While it is true that the former group focused on the effects of phenformin and the latter group on effects of 991, both agreed that the concentrations of biguanides they used caused AMPK activation, so discrepancies still seem to be present.

The evidence in favor of the mechanism proposed by Johanns et al. (2016) seems quite compelling, although they did not perform any studies in vivo. However, there is at least one puzzling feature of the mechanism proposed by Miller et al. (2013). Although they did not perform a detailed kinetic analysis of the inhibition of adenylate cyclase by AMP, the effect appeared to be competitive with the substrate ATP, because it was relieved as the concentration of the latter was increased. At 160 Î¼M ATP, maximal inhibition by AMP was > 90%, but at 1.3 mM ATP (which is at the low end of the range of normal cellular ATP concentrations) the maximal inhibition, even at 300â€“600 Î¼M AMP, was only about 30%. Thus, any inhibition of adenylate cyclase at normal cellular ATP levels would appear to be rather modest. In addition, despite the apparent competitive inhibition the exact binding site for AMP on adenylate cyclase remains undefined. Studies of an AMP-insensitive mutant, as has already been achieved for fructose-1,6-bisphosphatase (Section 5.2.4) would be a valuable confirmation of their hypothesis.

Note also that neither of these two mechanisms explains the acute inhibition by metformin of glucose production

induced by the cell-permeable cyclic AMP analog, dibutyryl cyclic AMP (Cao et al., 2014; Foretz et al., 2010), which bypasses adenylate cyclase and is also resistant to breakdown by phosphodiesterases. The effects observed in the latter studies must have other explanations, such as the inhibition of fructose-1,6-bisphosphatase by AMP (Section 5.2.4).

Whatever the exact mechanism(s) by which biguanides antagonize cellular cyclic AMP production, the effect could help to explain some of the acute effects of the drugs to inhibit hepatic gluconeogenesis, as well as their longer-term effects to inhibition expression of gluconeogenic genes. Many of the effects of increased cyclic AMP are mediated by activation of the cyclic AMP-dependent protein kinase (PKA), and several of the downstream targets of that kinase are involved in the regulation of gluconeogenesis, as summarized in Fig. 5. Starting with the acute effects, phosphorylation of 6-phosphofructo-2-kinase:fructose-1,6-bisphosphatase (PFKFB1) would cause a drop in levels of the regulatory metabolite fructose-2,6-bisphosphate, which otherwise promotes glycolysis by activating the glycolytic enzyme 6-phosphofructo-1-kinase, while depressing gluconeogenesis by inhibiting the gluconeogenic enzyme fructose-1,6-bisphosphatase (Rider et al., 2004). Indeed, phenformin treatment of mouse hepatocytes reduces phosphorylation of PFKFB1 at the PKA site in response to glucagon (Miller et al., 2013). PKA also phosphorylates and inactivates the liver isoform of pyruvate kinase (Fig. 5), further inhibiting glycolysis and promoting gluconeogenesis instead (Pilkis et al., 1988). Turning to the longer-term effects, PKA promotes transcription of the gluconeogenic enzymes phosphoenolpyruvate kinase (PEPCK) and glucose-6-phosphatase (G6Pase), in part via effects on the transcription factor CREB. Phosphorylation of CREB by PKA is involved in activating transcription of these genes (Altarejos and Montminy, 2011) and, consistent with this, Miller et al. (2013) found that phenformin inhibited CREB phosphorylation in mouse hepatocytes, while the AMPK activator 991 had similar effects in the study of Johanns et al. (2016). The regulation of these genes also involves phosphorylation of the transcriptional co-activators CRTC2 or CRTC3 (previously called TORC2/TORC3) as well as Class IIA histone deacetylases such as HDAC4, 5, 7 and 9, promoting their retention in the cytoplasm rather than the nucleus by enhancing their interactions with 14-3-3 proteins (Henriksson et al., 2015; Walkinshaw et al., 2013). While there is evidence that TORCs and HDACs can be phosphorylated by AMPK (Koo et al., 2005; Mihaylova et al., 2011), regulation of expression of the G6Pase gene can still occur in the complete absence of hepatic AMPK (Foretz et al., 2010). Phosphorylation of HDACs and CRTC2/3 can also be mediated by one or more of the AMPK-related kinases SIK1, SIK2 or SIK3, which have a closely related recognition motif to AMPK (Sonntag et al., 2017). SIK1, SIK2 and SIK3 are phosphorylated at multiple sites by PKA; this appears to repress SIK activity via a mechanism that, once again, involves binding of 14-3-3 proteins (Patel et al., 2014; Sonntag et al., 2018).

Reference

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