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Review

Unique roles of glucagon and glucagon-like peptides: Parallels in understanding the functions of adipokinetic hormones in stress responses in insects

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ABSTRACT

Glucagon is conventionally regarded as a hormone, counter regulatory in function to insulin and plays a critical anti-hypoglycemic role by maintaining glucose homeostasis in both animals and humans. Glucagon performs this function by increasing hepatic glucose output to the blood by stimulating glycogenolysis and gluconeogenesis in response to starvation. Additionally it plays a homeostatic role by decreasing glycogenesis and glycolysis in tandem to try and maintain optimal glucose levels. To perform this action, it also increases energy expenditure which is contrary to what one would expect and has actions which are unique and not entirely in agreement with its role in protection from hypoglycemia. Interestingly, glucagon-like peptides (GLP-1 and GLP-2) from the major fragment of proglucagon (in non-mammalian vertebrates, as well as in mammals) may also modulate response to stress in addition to their other physiological actions. These unique modes of action occur in response to psychological, metabolic and other stress situations and mirror the role of adipokinetic hormones (AKHs) in insects which perform a similar function. The findings on the anti-stress roles of glucagon and glucagon-like peptides in mammalian and non-mammalian vertebrates may throw light on the multiple stress responsive mechanisms which operate in a concerted manner under regulation by AKH in insects thus functioning as a stress responsive hormone while also maintaining organismal homeostasis.

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1. Introduction

Glucagon is a 29-amino acid peptide hormone processed from proglucagon and plays a critical role in glucose metabolism *in vivo* in vertebrates including humans. The prohormone proglucagon is expressed in various tissues (mainly in brain, pancreas and intestine) and is proteolytically processed into several peptide hormones in a tissue specific manner. In vertebrates, both pancreas and intestine are known to actively process the proglucagon gene, and release a mixture of peptides, including GLPs with distinct physiological functions (Mommsen, 2000). The functional glucagon-like peptides (GLP-1 and 2) are processed by subtilisin-like proprotein convertases (PC1-3) in intestinal cells (Rouille et al., 1997a) and into functional glucagon by PC2 in the pancreatic α cells (Rouille et al., 1994, 1997b). Glucagon acts via a seven-transmembrane G protein coupled receptor which for example in rats consists of 485 amino acids (Jelínek et al., 1993). Glucagon binding sites have been identified in a number of tissues including

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liver, brain, pancreas, kidney, intestine and adipose tissues (Christophe, 1995). There is also a large body of literature on glucagon as regulators of metabolism in fishes (reviewed by Mommsen and Plisetskaya, 1991; Plisetskaya and Mommsen, 1996). In general, in mammalian systems, glucagon is released into the blood stream when circulating glucose is low. It thus stimulates hepatic glucose output, thereby leading to increases in glycemia, which provides a counter regulatory mechanism to insulin in maintaining glucose homeostasis *in vivo* (Jiang and Zhang, 2003). This property of glucagon was instrumental in its nomenclature since it was identified as a compound contaminating pancreatic extracts that had quite the opposite effects of insulin (Kimball and Murlin, 1923). However, recent findings also indicate that glucagon actually increases energy expenditure (Habegger et al., 2010; Heppner et al., 2010) much like adipokinetic hormones (AKHs) in insects (Goldsworthy, 1994).

This is paradoxical since hypoglycemia is a state of energy deficiency, and glucagon actually increases energy expenditure, a situation which is quite in contrast to expectations. Glucagon-like peptides, as mentioned earlier, are one of several cleavage products from the precursor proglucagon. Two forms of GLP1, GLP-1 (7–37 amide) and GLP-1 (7–36 amide) are produced by the L-cells in the jejunum and colon in response to oral glucose or mixed meals (Creutzfeldt and Nauck, 1992). GLP-2 on the other hand, promotes growth of the intestinal mucosa (Estall and Drucker, 2006). GLP-1 increases glucose mediated insulin secretion by activating specific GLP-1 receptors on insulin secreting β cells in the pancreatic islets (Fehmann et al., 1994). The increase in insulin secretion is glucose dependent and originates from increases in cAMP and Ca^{2+} levels. This "incretin effect" of GLP-1 is one of its most important functions, however, in addition to this physiological function, GLP-1 has also been implicated in cardiovascular functions, such that, in the basal state, GLP-1 may inhibit contractility, but after cardiac injury GLP-1 has been shown to constantly increase myocardial performance both in experimental animals and patients (Nikolaidis et al., 2004a,b; Bose et al., 2005; Nikolaidis et al., 2005). GLP-1 also possesses neurotropic effects and has also been suggested as a therapeutic agent for neurodegenerative diseases (During et al., 2003; Perry and Greig, 2004). Also, while the functions of GLP-1 in fishes have been elucidated in some detail (Mommsen, 2000), yet, the biological actions of GLP-2 remain unclear in them. (For a comprehensive review of the glucagon-like peptides and the physiology of glucagon-like peptide-1 please see Kieffer and Habener, 1999; Holst, 2007). The stress responsive roles and modes of action of glucagon and GLPs could probably stem from a more generalized physiological response to stress as was also described in the case of AKH in insects (Kodrík, 2008), which is analogous to glucagon in mammals.

In insects, metabolism and particularly the generation of energy are regulated by AKHs, small neuropeptides belonging to the adipokinetic hormone/red pigment concentrating hormone (AKH/RPCH) peptide family (Gäde et al., 1997; Kodrík, 2008), which are synthesized, stored and released by neurosecretory cells of the *corpora cardiaca* an endocrine gland connected to the brain. AKHs predominantly perform roles as stress responsive hormones by stimulating catabolic reactions (mobilization of lipids, carbohydrates, and amino acids) which generate more energy while simultaneously inhibiting their synthesis (Kodrík, 2008). This they do by mobilizing the entire energy reserves to counter immediate stress situations while suppressing processes that would divert energy elsewhere, such as synthesis reactions. AKHs are usually octa or deca peptides but other forms of AKH have also been reported (Köllisch et al., 2000; Gäde et al., 2006). They possess a pyroglutamate residue blocking the N-terminus and amide group blocking the C-terminus. The amino acids tryptophan and glycine are at position 8 and 9 (when present); in addition to tryptophan the AKH molecule contains at least one more aromatic amino acid, most commonly phenylalanine at position 4 (Gäde et al., 1997). The insect fat body, an organ analogous to the vertebrate liver, is the main site of action of AKHs. The homeostatic regulation of blood sugar levels is a fundamental physiological process in both vertebrates and invertebrates. The fundamental endocrine regulation of homeostatic blood sugar levels is conserved in insects for e.g. an insulin-related peptide, bombyxin, lowers hemolymph sugar concentrations in a dose-dependent manner in the silkworm *Bombyx mori* and transgenic ablation of *dilp*-producing neurons results in the elevation of total blood sugar (Satake et al., 1997; Lee and Park, 2004). In the fruitfly *Drosophila*, insulin- and glucagon-like peptides have been reported and they are represented by seven *Drosophila* insulin-like peptides (DILPs), (Brogiolo et al., 2001; Cao and Brown, 2001) and AKH respectively (Wu and Brown, 2006). Moreover, dysregulation of glucose homeostasis is also observed when *akh*-expressing cells are ablated (Kim and Rulifson, 2004; Lee and Park, 2004). Also, microarray analysis of flies in which insulin-producing cells (IPCs) were ablated revealed a target gene, *target of brain insulin* (*tobi*), encoding an evolutionarily conserved α-glucosidase. *tobi* expression is increased by dietary protein and decreased by dietary sugar, which is reminiscent of mammalian glucagon secretion which also functions in a similar manner, and this suggests that *tobi* is regulated by a glucagon analog i.e. AKH. These findings strongly suggest that the insulin–glucagon system of mammals and the DILP-AKH system of *Drosophila* may have analogous roles in regulating metabolism (Buch et al., 2008). Also, similar to glucagon, AKHs mechanism of action includes signal transduction through membrane receptors linked to G-protein that activates multiple pathways leading to production of energy providing substrates such as trehalose, diacylglycerol or proline. Thus, at the functional level, AKHs resemble glucagon, whose main function (like AKH) is to mobilize energy reserves, mainly glucose and participation in glucose homeostasis in the blood. Despite an obvious lack of structural similarity, this functional similarity points to a wealth of knowledge one can obtain about the unique roles of glucagon and GLPs from emerging studies on AKHs in insect model systems and vice versa. This review focuses on studies on insect AKHs as well as parallel studies on glucagon and GLPs in higher animals which informs us and suggests a close functional similarity between the two stress responsive hormones, which perform similar unique roles in these two very different, yet physiologically comparable systems in an effort to maintain organismal homeostasis.

2. Glucagon and GLPs in insects

Since both insect AKHs and vertebrate glucagon perform similar functions, attempts have been made to discover glucagon or GLPs in insects and also to seek a role for mammalian glucagon in insect systems. Presence of a substance with hyperglycemic activity comparable to glucagon was first reported by Steele (1961, 1963) in the *corpus cardiacum* of *Periplaneta americana*. These observations were confirmed by further studies on this cockroach (Ralph and McCarthy, 1964; Brown, 1965; Natazili and Frontali, 1966; Natazili et al., 1970), and extended to many other insects: cockroaches *Blaberus discoidalis* (Bowers and Friedman, 1963) and *Leucophaea maderae* (Wiens and Gilbert, 1967); the locust *Locusta migratoria* (Goldsworthy, 1969; Highnam and Goldsworthy, 1972); the black blowfly *Phormia regina* (Friedman, 1967), the blowfly *Calliphora erythrocephala* (Normann and Duve, 1969; Vejbjerg and Normann, 1974; Normann, 1975); the bee *Apis mellifera* (Natazili and Frontali, 1966) and the moth *Manduca sexta* (Tager et al., 1975). All these earlier articles which were published before the isolation and identification of the first insect peptide hormones did not differentiate between glucagon and GLP effects, and the possible effect of AKH. On the other hand an immunochemically similar GLP was reported from the hemolymph of *M. sexta* (Kramer et al., 1980). However despite its structural similarity to vertebrate glucagon and some contradictory reports as to its influence on mobilization of energy reserves in insects (Tager et al., 1976; Ziegler, 1979), an unambiguous answer to the question of its actual role in insects was not forthcoming.

We demonstrated in our earlier paper (Alquicer et al., 2009) an immunological presence of glucagon in insect organs (gut, CNS) and suggested that glucagon may have a unique role in insects much like AKH which functions to ameliorate response to stress. The study also