The Glucagon–Miniglucagon Interplay

A New Level in the Metabolic Regulation

DOMINIQUE BATAILLE, GHISLAINE FONTÉS, SAFIA COSTES, CHRISTINE LONGUET, AND STÉPHANE DALLE

INSERM U 376, CHU Arnaud-de-Villeneuve, 34295 Montpellier Cedex 05, France

ABSTRACT: Miniglucagon (glucagon 19–29) is the ultimate processing product of proglucagon, present in the glucagon-secreting granules of the α cells, at a close vicinity of the insulin-secreting β cells. Co-released with glucagon and thanks to its original mode of action and its huge potency, it suppresses, inside the islet of Langerhans, the detrimental effect of glucagon on insulin secretion, while it leaves untouched the beneficial effect of glucagon on glucose competence of the β cell. At the periphery, miniglucagon is processed at the surface of glucagon- and insulin-sensitive cells from circulating glucagon. At that level, it acts *via* a cellular pathway which uses initial molecular steps distinct from that of insulin which, when impaired, are involved in insulin resistence. This bypass allows miniglucagon to act as an insulin-like component, a characteristic which makes this peptide of particular interest from a pathophysiological and pharmacological point of views in understanding and treating metabolic diseases, such as the type 2 diabetes.

KEYWORDS: Miniglucagon; glucagon; proglucagon processing; insulin; type 2 diabetes

INTRODUCTION

The control of the glucose metabolism relies on both regulatory hormones (mainly insulin) and counterregulatory hormones (such as glucagon). From glucagon, is produced another peptide, miniglucagon or glucagon (19–29), which acts against its mother-hormone, and adds to the metabolic regulation novel steps that are to be taken into account in understanding (and possibly treating) metabolic diseases, such as type 2 diabetes.

Address for correspondence: Dominique Bataille, INSERM U 376, CHU Arnaud-de-Villeneuve, 34295 Montpellier Cedex 05, France. Voice: +33 4 67 41 52 30; fax: +33 4 67 41 52 22.
e-mail: bataille@montp.inserm.fr

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PROGLUCAGON

Proglucagon[1] is the archetype of a multifunctions precursor,[2] which by tissue-specific posttranslational processing, furnishes the organism with signal molecules implicated in various regulatory mechanisms. In contrast to intestinal L cells that produce oxyntomodulin, glicentin, GLP-1, and GLP-2, the type of processing present in the endocrine pancreas, which appeared probably late during evolution, isolates the glucagon sequence from the octapeptide common to oxyntomodulin and glicentin, completely changing its biological specificity. However, glucagon is not the single peptide produced from proglucagon in α cells, since a further step in processing liberates a peptide displaying original and unexpected, if not surprising, properties.

MINIGLUCAGON

This peptide corresponds to the 11-amino acid C-terminal fragment of glucagon (glucagon 19–29). Its existence was uncovered[3] as being the peptide responsible for the observed glucagon effect on the hepatocyte plasma membrane calcium pump.[4] Since that time, we have observed that it is produced from circulating glucagon (few percent is transformed during a pass through an organ) by an enzymatic system at the level of the Arg_{17}-Arg_{18} basic doublet at the surface of glucagon-sensitive target cells.[5] Immediately after its local production and action, it is very quickly cleared from circulation. Accordingly, this peptide, carried to its target tissues by its mother hormone glucagon, is not a hormone itself and may act only locally, by modulating the actions of the mother hormone.

In those (glucagon- and miniglucagon-sensitive) tissues, it displays biological effects at extremely low doses (picomolar or lower), at least three order of magnitude below the active doses of glucagon.[6] The amount of miniglucagon produced (3–4% of the glucagon concentrations in α cells, a similar proportion in peripheral target tissues) is largely compensated by the huge difference between the active doses of the respective peptides. Miniglucagon is cosecreted with glucagon under hypoglycemic physiological conditions.[7]

Miniglucagon blocks the effect of all insulin secretagogues that use calcium entry into β cells, such as glucose, glucagon, GLP-1, or sulfonylurea.[6,7] It acts through a repolarization of the β cell plasma membrane, closing the voltage-sensitive calcium channels whose opening is necessary for secretion, while leaving untouched the cyclic adenosine 3′,5′-monophosphate (cAMP) levels.[6]

It is produced both in the glucagon secretory granules and at the surface of target tissues by the miniglucagon-generating endopeptidase (MGE), recently identified[8] as a metalloendoprotease (NRDc) that cleaves at the N-terminus

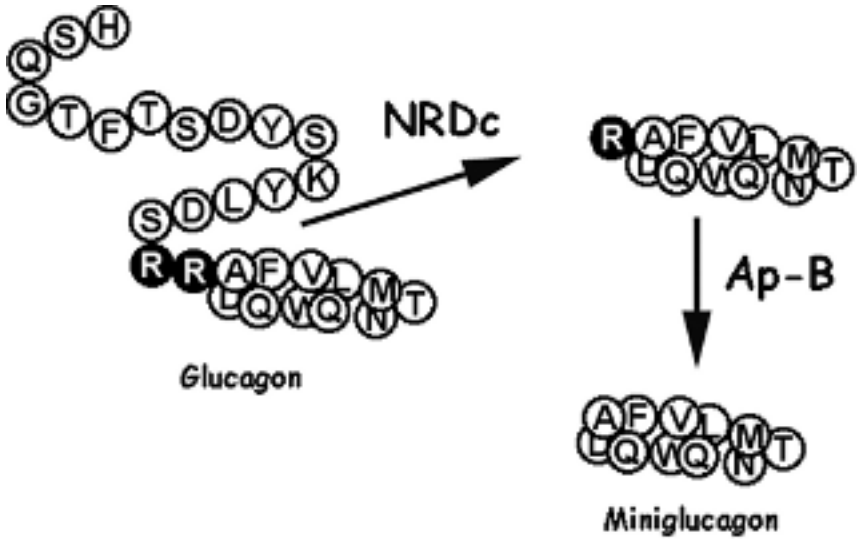

FIGURE 1. Processing of glucagon into miniglucagon by N-arginine dibasic convertase (NRDc) and aminopeptidase-B (Ap-B).

of arginine residues followed by suppression of the remaining basic residue(s) by a specific aminopeptidase (FIG. 1).

GLUCAGON–MINIGLUCAGON RELATIONSHIPS INSIDE THE ISLET

It is noteworthy that a *bona fide* partnership exists between α and β cells thanks to the fact that 35–50% of the latter have DIRECT contacts with α cells.[9] During the interprandial state when the hyperglycemic action of glucagon is necessary, the cosecreted miniglucagon "turns the calcium tap off" in the β cell, rendering impossible any nonphysiological effect of glucagon. This explains that, while exogenous glucagon stimulates insulin release,[10] endogenous glucagon, fortunately does not.[11] It remains to explain why glucagon has receptors at the surface of the β cell.[10] The glucagon receptors are coupled to adenylate cyclase, the cAMP produced stimulates protein kinase A (PKA) that activates molecules implicated in the trafficking of secretory granules, as well as nuclear targets which control gene expression necessary for regulating the secretory machinery. Glucose competence, that is, the ability to respond properly to glucose, may be maintained, or even recovered when partially lost, by peptides, such as glucagon,[12] that increase the cyclic AMP–PKA pathway in the β cell. Recent data[13,14] strongly suggest that the p44/42 MAP kinases (ERK1/2) are implicated in those mechanisms, since they activate nuclear

transcription factors which regulate genes necessary for the β cell survival and functions, such as the antiapoptotic factor Bcl-2 or proinsulin. Furthermore,[14] ERK 1/2 phosphorylate proteins present at the surface of secretory granules, favoring the insulinosecretory effect of the awaited postprandial glucose wave (glucose competence). Since glucagon is able to stimulate ERK 1/2 in the β cell (13) in a pure cAMP/PKA manner, thus independently of calcium entry, it may exert its beneficial actions on the neighboring β cells (maintenance of the β cell mass and glucose competence) during the interprandial states, actions untouched by miniglucagon.

GLUCAGON–MINIGLUCAGON RELATIONSHIPS AT THE PERIPHERY

Secreted glucagon is transported to its target tissues *via* circulation. In contrast, its very short half-life precludes a hormonal status for miniglucagon. Thus, any observed effect of miniglucagon on a tissue remote from the islets is on account of a local production from circulating glucagon through MGE present at the cell surface. The possible importance of miniglucagon at the periphery was recently supported by the observation that a perfusion of the peptide together with a glucose load in vigil rats leads to a smaller insulin response, without any change in glycemia.[15] These observations led us to hypothesize the existence of an effect of the peptide on peripheral glucose-utilizing tissues, favoring or mimicking insulin action. We observed[15] that miniglucagon, as insulin does, induces translocation of the Glut-4 glucose transporter to the

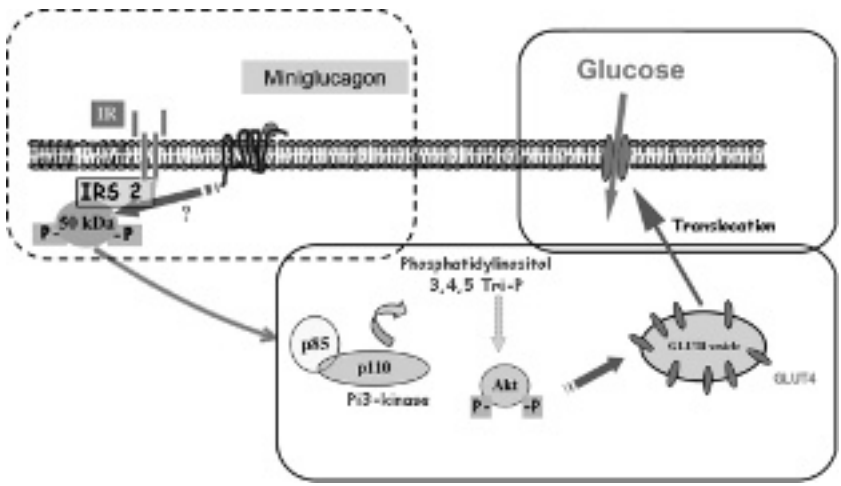

FIGURE 2. Mode of action of miniglucagon on glucose transport in 3T3-L1 adipocytes. The plain frames highlight the pathways common with insulin, and the dotted frame the original pathway.

plasma membrane of 3T3 adipocytes by use of the distal steps used by insulin (PI_3-kinase and Akt/PKB). On the other hand, the early steps differ: instead of using phosphorylation of the insulin receptor (IR) and of the insulin receptor substrate-1 (IRS-1), the miniglucagon action relies on phosphorylation of a 50-kDa protein that forms with the nonphosphorylated IR/IRS-2 (and not IRS-1) complex a signaling platform that passes the miniglucagon message to the PI_3-kinase/Akt/Glut-4 pathway (FIG. 2). Since a similar mechanism exists in the muscle (unpublished observations), miniglucagon appears as an insulin partner, acting in a direction opposite to that of its mother hormone, glucagon. Accordingly, any change in the MGE activity at the surface of target cells will set differently the glucagon–miniglucagon balance and, as a consequence, the glucagon versus insulin relationship, of major importance in regulating the catabolic–anabolic balance. The secondary processing of glucagon into miniglucagon appears thus as a new level in the metabolic regulation, which should be taken into account in the understanding of metabolic diseases, such as type 2 diabetes.

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