Regulation of metabolism by amino acid dependant signal transduction

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Summary

Although amino acids are known to be building blocks of proteins and substrates for gluconeogenesis and urea synthesis, they also play an important role in the regulation of metabolism. One process that is controlled by amino acids is the lysosomal proteolytic pathway also known as autophagy. Autophagy is accelerated during starvation when concentrations of amino acids are low, and is inhibited in the fed state when concentrations of amino acids are high. During autophagy a small part of the cytoplasm is surrounded by a double isolation membrane leading to the formation of a vesicle (the autophagosome) which fuses with a lysosome so that degradation of the engulfed material can occur. A detailed description of the autophagic process is given in chapter 1. The control of autophagy by amino acids is related to the activation of the mTOR/p70S6 kinase/S6 signalling pathway. Both the inhibition of autophagy and the stimulation of signal transduction by amino acids is potentiated by insulin (see below, chapter 5).

As described in chapter 2, downregulation of the PI 3-kinase/PKB pathway, by the overexpression of the tumor suppressor PTEN, led to activation of autophagy in HT-29 cells, a colon cancer cell line. In line with this result was the inhibitory effect of PKB overexpression on autophagy. PKB is an oncogenic protein that is frequently overactivated in cancer cells. It appeared that there is a connection between the control of a major catabolic route, autophagy, and that of a signaling pathway frequently altered in human cancers.

In hepatocytes, another metabolic pathway that is controlled by amino acids is the synthesis of glycogen from glucose. Certain amino acids (e.g. glutamine and proline) cause cell swelling due to their concentrative Na⁺-dependent transport. In response to the initial cell swelling, hepatocytes undergo “regulatory volume decrease” (RVD) and release KCl in an attempt to restore their original volume. This decrease in chloride concentration is in part responsible for the stimulation of glycogen synthesis from glucose by amino acids, because the fall in intracellular chloride results in de-inhibition of glycogen synthase phosphatase. In the literature it was assumed that PI 3-kinase is involved in the stimulation of glycogen synthesis by amino acids, because of inhibition of glycogen synthesis by the PI 3-kinase inhibitors LY294002 and wortmannin. In chapter 3 we show that in hepatocytes activation of PI 3-kinase may not have been responsible for the stimulation of glycogen synthesis by amino acids. A complicating factor with regard to the use of PI 3-kinase inhibitors is that these compounds also inhibit autophagic proteolysis because of the requirement of PI 3-kinase class III for autophagy. Therefore, we think that reported inhibition of glycogen synthesis by PI 3-kinase inhibitors may have been indirect, i.e. by inhibition of the production of amino acids from intracellular proteins. This view was supported by our finding that specific inhibitors of proteolysis that do not affect PI 3-kinase also inhibited glycogen production under basal conditions.

As mentioned before, amino acids can stimulate a signal transduction pathway resulting in the phosphorylation of mTOR-downstream targets as p70S6 kinase. It is generally accepted that mTOR functions as an amino acid sensor and recently it was proposed that mTOR might also
act as an ATP sensor. The data described in chapter 4 suggest that the AMP-activated protein kinase (AMPK) is an additional element in the control of mTOR-dependent signalling. In hepatocytes, amino acid-dependent stimulation of the mTOR/p70S6 kinase pathway was inhibited upon stimulation of AMPK by the AMP analog AICAR (mimicking energy-low conditions). Furthermore, it was found that lowering intracellular ATP concentrations by addition of either glycerol or fructose also inhibited amino acid-dependent signalling.

Chapter 5 describes the interaction between amino acids and insulin in stimulating the PI-3 kinase/PKB/mTOR/p70S6 kinase pathway in rat hepatocytes. It was found that high concentrations of amino acids (mimicking the fed state) stimulate p70S6 kinase activity maximally, whereas low concentrations of amino acids (mimicking starvation) only slightly stimulate p70S6 kinase activity. In combination with insulin, low concentrations of amino acids were as efficient in stimulating p70S6 kinase as high concentrations of amino acids. By contrast, insulin alone had no effect on p70S6 kinase activation. Amino acids were unable to stimulate PI 3-kinase and PKB, two proteins that were activated by insulin. Experiments with inhibitors of PI 3-kinase and PKB led us to the conclusion that activation of the PI 3-kinase/PKB pathway by insulin is required for full activation of mTOR-mediated p70S6 kinase by low concentrations of amino acids, but that PKB activity is not needed for the activation of p70S6 kinase by high concentrations of amino acids. Because amino acids proved to be unable to stimulate PI 3-kinase and because amino acid-dependent stimulation of p70S6 kinase was sensitive towards PI 3-kinase inhibitors we concluded that basal PI 3-kinase activity is probably needed for full activation of p70S6 kinase by amino acids.

Among the various amino acids, leucine is most effective in stimulating the mTOR/p70S6 kinase pathway. However, for full activation of the pathway the action of other amino acids was needed. The ability of these other amino acids (e.g. glutamine and proline) to promote cell swelling probably explains their synergy with leucine, as is demonstrated in chapter 6. Glutamine and proline induced cell swelling which resulted in a fall in intracellular chloride. This fall in chloride may be involved in the regulation of AMPK and of glutamate-dependent protein phosphatase (GAPP), two proteins that are known to control amino acid-dependent activation of the mTOR/p70S6 kinase pathway. Another enzyme that may be regulated by chloride is aminoacyl-tRNA synthetase. Indications from the literature suggest that free, uncharged tRNA may act as an amino acid sensor that influences the activity of mTOR. In line with this hypothesis is the outcome of experiments described in chapter 6. In hepatocytes incubated with aminoalcohols, inhibitors of the activity of aminoacyl-tRNA synthetases, amino acid-dependent activation of the mTOR/p70S6 kinase pathway was strongly inhibited. Leucinol was particular effective in this regard. The mechanism responsible for the potentation of the leucine-dependent activation of the mTOR/p70S6 kinase pathway by cell swelling may involve a decrease in intracellular chloride due to RVD, which subsequently stimulates the activity of GAPP and/or aminoacyl tRNA synthetases.

The results described in this thesis support the view that amino acids are potent stimulators of a signal transduction pathway that is also used by insulin. The mechanism by
which amino acids stimulate the mTOR/p70S6 kinase pathway remains to be elucidated. In chapter 7 possible mechanisms are proposed on the basis of the work described in this thesis and also on the basis of recent literature. In this chapter, the importance of amino acid-dependent signalling in diabetes, cancer and ageing is also discussed.