GHRELIN AND DES-ACYL GHRELIN INHIBIT ISOPROTERENOL-INDUCED LIPOLYSIS IN RAT ADIPOCYTES THROUGH ACTIVATION OF PHOSPHODIESTERASE 3B

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The gastrointestinal octanoylated peptide ghrelin is a pleiotropic hormone, exerting both local and systemic actions. Ghrelin is involved in the maintenance of metabolic homeostasis, in fact it stimulates food intake via central mechanisms and increases adiposity through adipogenetic and antilipolytic effects that take place in peripheral tissues. We have previously demonstrated that ghrelin antagonizes isoproterenol-induced lipolysis in rat adipocytes that did not express the cloned ghrelin receptor GHS-R1a. Similarly did the major circulating isoform, des-acyl ghrelin, which does not bind GHS-R1a, but shares a common high-affinity binding site with ghrelin on the adipocytes. The molecular identity of such receptor remains unknown, as well as related downstream signaling pathways in the adipocytes. In this study we investigated the signaling events originating from the receptor for ghrelin and des-acyl ghrelin which are responsible for the antilipolytic effect on isolated rat adipocytes exposed to isoproterenol. Ghrelin and des-acyl ghrelin had no effect on glycerol release in absence of lipolytic stimuli, however both reduced isoproterenol-stimulated release of glycerol depending on extracellular Ca^{++} availability. It is known that cAMP is necessary to activate PKA, which in turn phosphorylates hormone sensitive lipase (HSL), causing lipolysis. Ghrelin and des-acyl ghrelin were capable of counteracting the lipolysis triggered by the rise on intracellular cAMP, independently from the causing events, was it ß adrenergic-receptors activation or forskolin stimulation of adenylyl cyclase. Additionally, exogenous re-introduction of 8-Br-cAMP reverted ghrelin and des-acyl ghrelin effect, indicating that cAMP is a major target for their anti-lipolytic effect. The use of specific inhibitors of intracellular mediators allowed the identification of some effector proteins involved: both ghrelin and des-acyl ghrelin ability to prevent lipolysis was impaired upon blockade of any of the following enzymes, PI3K, AKT 1/2 and phosphodiesterase 3B (PDE3B), suggesting that one mechanism by which the receptor for ghrelin and des-acyl ghrelin impairs lipolysis, is opposing cAMP accumulation through stimulation of PDE3B-mediated cAMP conversion into AMP. A small effect, although significant, was found for ERK 1/2 inhibitors in preventing ghrelin and des-acyl ghrelin activity, while no effect at all was found for PKC inhibitors, indicating that ERK may have a marginal role in inhibiting lipolysis, but not PKC. Studies are in progress to verify if alternative pathways may be triggered by ghrelin and des-acyl ghrelin, leading to similar anti-lipolytic effect when the lipolysis is induced by different stimuli involving direct stimulation of HSL.