PROJECT REVIEW

“A fair competition for the growth hormone secretagogue receptor”

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Growth hormone secretagogues (GHS) are molecules that stimulate the secretion of human growth hormone from the pituitary. They have proven to be potent agonist and as they have been developed by pharma even before the natural ligand or receptor were known, GHS display large structural heterogeneity. In order to address the entire family of molecules one can only target what all GHS have in common: the interaction with the receptor. Based on this premise we developed a competition assay and have demonstrated already its functionality.

Within the context of this project the assay validation shall be performed as well as further development of improvements to facilitate implementation in other anti-doping laboratories.

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Result and Conclusion:

Growth hormone secretagogues (GHS) are a large group of, structurally very diverse, chemical compounds that all share the interaction with the growth hormone secretagogue receptor 1a (GHSR-1a). Following the interaction of GHS with GHSR-1a, located in the pituitary gland, the release of growth hormone (GH) is stimulated through the intracellular Ca2+-release mechanism that is completely independent from the cAMP mechanism of the growth hormone releasing hormone. Both the receptor GHSR-1a as the natural ligand (ghrelin) were discovered less than 20 years ago whereas the development of GHS was initiated more than 30 years ago giving rise to large structural heterogeneity in the pharmacophores. Within the framework of this project we have developed a universal screening method for all GHS, irrespective of the chemical nature and structural characteristics, by using the single feature that all share: the interaction with GHSR-1a. We have established a stable and recombinant expression of this receptor and use this in combination with 125I-ghrelin to establish a 100% binding situation. The co-incubation with a processed urine extract (from 2.5 ml urine for a triplicate measurement) indicates the presence of a secretagogue if binding falls below an established threshold.

Following the development of the protocol we established the threshold value and limit of detection, the functional assay sensitivity, analytical stability, and intra- and interassay accuracy. We have assessed the influence of ethnicity, gender, age, and exercise, finding no significant influence of any of these factors on the assay readouts. Finally, we evaluated specimens from a pralmorelin administration study and compared the results to those obtained from analysis by LC-MS. We found that for the intact compound both protocols provided very similar results.