Fine Tuning of the Luteinizing Hormone Receptor Signaling Example of Natural and Allosteric Bias

The activity of two hormones, the luteinizing (LH) and human chorionic gonadotropin (hCG) is mediated by the LH**/**CG receptor (LH/CGR). This receptor is a G protein-coupled receptor (GPCR) known for its central role in both male and female reproduction. Thus, a particular interest has been given to this receptor with regards to infertility and contraception (LH) and maintenance of pregnancy (hCG), and various disorders of the reproductive system. Although biased signaling has long been recognized as a nuance of function and regulation of GPCRs, it has only recently emerged as a reality of gonadotropin (GN) signaling. Here we describe two examples of biased signalling at LH/CGR involving the two natural human gonadotropins, hCG and hLH, as well as two small molecule negative allosteric modulators (NAMs). Thus, for many years LH and hCG were considered biologically equivalent because they bind to the same receptor and share partial primary sequence identity. Here evidence is presented that demonstrates LH/CGR is able to differentiate the downstream intracellular signaling and trafficking of these two human gonadotropins. The observed biased signaling was translated into different steroidogenic profiles (progesterone and testosterone production) under hCG and hLH stimulation of murine Leydig tumor (mLTC-1) cells endogenously expressing LHR. The significance of this physiological bias may have implications when hCG is expressed during pregnancy but hLH is suppressed by luteal progestin. The biased signaling was confirmed using two small molecules, ADX68692 and ADX68693, initially reported as NAMs for the closely related follicle-stimulating hormone receptor (FSHR). The two compounds also allosterically act on LH/CGR with differential antagonistic profiles on cAMP (Log IC_{50} of -4.85 ± 0.36 and -5.85 ± 0.14 for ADX68692 and ADX68693, respectively) and ß-arrestin (Log IC_{50} of -6.02 ± 0.11 and -6.82 ± 0.11 for ADX68692 and ADX68693, respectively) pathways in HEK293 cells. In addition, the two NAMs presented different antagonism of LHR evidenced by differential progesterone and testosterone production in mLTC-1 cells (*Bias factor = 6.18*). Together, these data illustrate natural orthosteric and pharmacological allosteric biases with implications in the development of drugs to control of human reproduction and the related public health issues.