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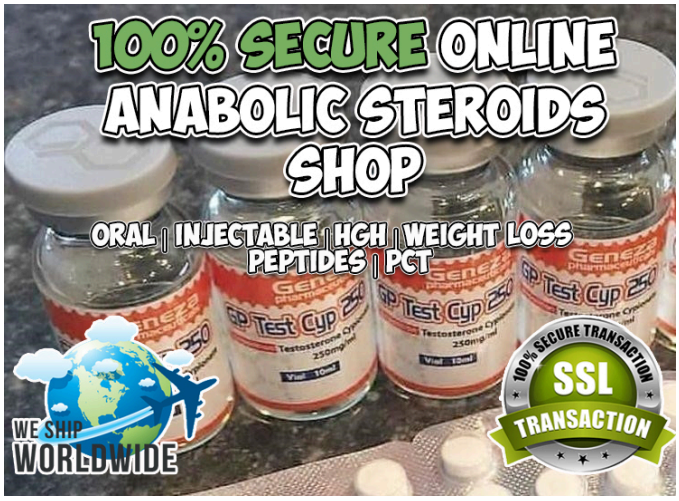

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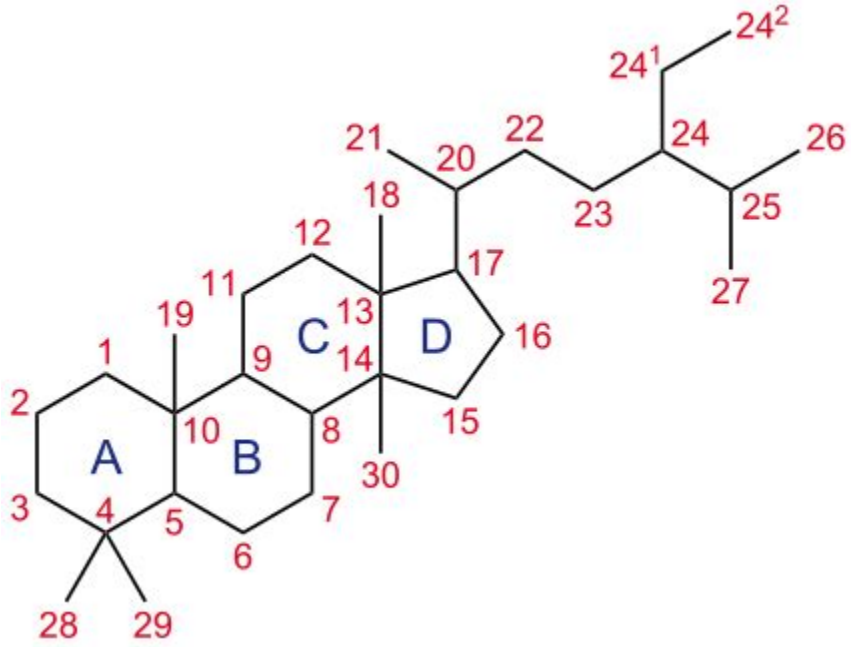

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British Journal of Pharmacology (2004) 143, 626–637
www.nature.com/bjp

The melanocortin receptor subtypes in chicken have high preference to ACTH-derived peptides

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1 Melanocortin (MC) receptors are widely distributed throughout the body of chicken, like in mammals, and participate in a wide range of physiological functions.

2 To clarify the pharmacological impact of ligands acting in the MC system, we expressed the chicken MC1, MC2, MC3, MC4 and MC5 (cMC1–5) receptors in eukaryotic cells and performed comprehensive pharmacological characterization of the potency of endogenous and synthetic melanocortin peptides.

3 Remarkably, the cMC receptors displayed high affinity for ACTH-derived peptides and in general low affinity for α-MSH. It is evident that not only the cMC2 receptor but also the other cMC receptors interact with ACTH-derived peptide through an epitope beyond the sequence of α-MSH.

4 The synthetic ligand MTII was found to be a potent agonist whereas HS024 was a potent antagonist at the cMC4 receptor, indicating that these ligands are suitable for physiological studies in chicken.

5 We also show the presence of prohormone convertase 1 (PC1) and PC2 genes in chicken, and that these peptides are coexpressed with preopiomelanocortin (POMC) in various tissues.

British Journal of Pharmacology (2004) **143,** 626–637. doi:10.1038/sj.bjp.0705900

Keywords: GPCR; MSH; ACTH; MC receptor subtypes

Abbreviations: ACTH, adrenocorticotropic hormone; c, chicken; GPCR, G-protein-coupled receptor; HEK-293 cells, human embryonic kidney cell line, strain 293; HS024, cyclic MSH analogue, Ac-Cys-Nle-Arg-His-D-Nal(2)-Arg-Trp-Gly-Cys-NH2, disulphide bridge Cys1-Cys9; MC receptor, melanocortin receptor; MSH, melanocyte-stimulating hormone; MTII, cyclic MSH analogue, Ac-Nle4-c[Asp5, D-Phe7, Lys10]α-MSH (4–10)-NH2; MYA, million years ago; NDP-MSH, (Nle[4], D-Phe[7]) α-MSH; PC, prohormone convertase; POMC, proopiomelanocortin; QTL, quantitative trait locus; RT–PCR, reverse transcription PCR

Introduction

The domestic chicken (*Gallus gallus*) is among the most investigated non-mammalian vertebrate species (Cheng, 1997). It is likely that the use of chicken as an experimental model will increase, as its genome today is completely sequenced (Burt & Pourquie, 2003). Today, the human, mouse and rat genomes as well as the genomes from the teleost fishes fugu and zebrafish have been sequenced. The chicken genome will bridge the large evolutionary gap between mammals and fish. Moreover, there is a great interest in using chicken as a model for tracking quantitative trait locus (QTLs). For example, there are large programmes in place to track genes involved in regulation of body weight homeostasis using anorectic chickens (Carlborg *et al*., 2003).

G-protein-coupled receptors (GPCRs) are probably the most pursued group of target proteins for drug development. The melanocortin (MC) receptors are GPCRs that are involved in a wide range of physiological functions, including skin pigmentation, stress and immune response, sexual behaviour, cardiovascular regulation and energy homeostasis. The melanocortin peptides, including α-, β-, γ-melanocyte-stimulating hormone (MSH) and adrenocorticotropic hormone (ACTH), are generated in mammals through a series of ordered proteolytic cleavages of the precursor glycoprotein proopiomelanocortin (POMC). Two kinds of endoproteases are involved in this process, the prohormone convertase 1 (PC1; also called PC3) that generates ACTH from POMC and the prohormone convertase 2 (PC2) that cleaves ACTH and other parts of POMC to produce α-, β- and γ-MSHs. The main source of circulating melanocortins is the pituitary. POMC is primarily processed into ACTH in the anterior lobe, while the intermediate lobe is the major source of α-MSH (Adan & Gispen, 1997). The intermediate lobe is well developed in lower vertebrates, but in human adults and chicken it is difficult to detect α-MSH and the quantities of circulating MSH is, in general, low in these species. Tetrapods, including chicken, have five MC receptor subtypes (Takeuchi *et al*., 1996; 1998; Takeuchi & Takahashi, 1998a, b). The MC1 receptor is expressed in mammalian melanocytes where it regulates pigmentation. Mutations of the MC1 receptor in chicken,

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Advance online publication: 4 October 2004