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The melanocortin peptide HP228 displays protective effects in acute models of inflammation and organ damage

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Abstract

The clinically efficacious melanocortin peptide HP228 has here been investigated for its anti-inflammatory efficacy. In this study we have investigated the efficacy of HP228 in murine acute models of inflammation and myocardial ischaemia. Systemic treatment of mice with HP228 inhibited neutrophil accumulation in zymosan; urate crystal and carrageenan induced inflammatory models. In the urate model this was due to inhibition of pro-inflammatory chemokines and cytokines, whilst different mechanisms exist for zymosan peritonitis and carrageenan-induced air-pouch inflammation. HP228 was next evaluated in a model of myocardial ischaemia, another condition where cytokines and neutrophils are thought to play a causal role. HP228 caused a 50% reduction in myocardial damage following reperfusion. HP228 therefore inhibits the most important facet of the host inflammatory response namely leukocyte migration. These data show for the first time that the clinically efficacious peptide HP228 displays protective effects in models of inflammation and organ damage.

Keywords: Melanocortin; Cytokine; Chemokine; Inflammation; Myocardial ischaemia

1. Introduction

Melanocortin peptides are derived from a larger precursor called the pro-opiomelanocortin (POMC) protein and are characterised by a common amino acid motif (HFRW). Post-translational processing of the POMC protein leads to the generation of the melanocortins as well as β-lipotrophin, γ-lipotropin and β-endorphin (Catania et al., 2004). These peptides are little changed throughout evolution and can be traced back to the first vertebrates (Lipton and Catania, 1997). In the middle of the last century the parent adrenocorticotropic (ACTH) hormone was found to be clinically effective for the treatment of rheumatoid arthritis by Phillip Hench at the Mayo clinic (Hench et al., 1950) and in gouty arthritis (Gutman and Yu, 1950). However, due to secondary adrenal gland suppression its clinical use is quite restricted. Therefore ACTH sequences, which do not activate the hypothalamic–pituitary–adrenal (HPA) axis, have been investigated and used clinically in a multitude of disease pathologies. The $ACTH_{4-9}$ analogue (ORG2766) was shown to be beneficial in children with intractable seizures (Pentella et al., 1982) and in Alzheimer's disease where it prevented deterioration (Partanen et al., 1986). $ACTH_{4-10}$ improves focussing attention (Smolnik et al., 1999), whilst the synthetic peptide MTII was found to improve penile erection and sexual motivation (Wessells et al., 2000). The synthetic peptide HP228 which contains the $ACTH_{4-10}$ region has been shown to be a pan-agonist activating the $MC_{3/4}$ (Hruby et al., 1993), produced a significant analgesia without associated respiratory depression (Weinger et al., 1998). Therefore the identification of melanocortin peptides that are efficacious in disease pathologies but devoid of the side effects associated with adrenal gland suppression is of benefit.

To this end the last decade has focussed on identifying the molecular targets where the melanocortin peptides exert their biological effects. These belong to a group of seven transmembrane domain G-protein coupled receptor, termed melanocortin

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receptors they are termed melanocortin MC_1 receptor to melanocortin MC_5 receptor and agonism leads to adenylate cyclase activation and increases in intracellular cAMP (Wikberg et al., 2000). These receptors are expressed within the periphery and the central nervous system. To date melanocortin peptides, (e.g. α-melanocyte stimulating hormone, α-MSH) have been shown to possess a multitude of actions including modulation of the host inflammatory response in acute and chronic inflammation, including experimental bowel disease, allergy, and chronic (mycobacterium-induced arthritis) and systemic inflammation (endotoxemia) (Richards and Lipton, 1984; Ceriani et al., 1994). This inhibitory effect in inflammation has been demonstrated to be due to inhibition of cytokine synthesis and release from target cells in vitro (Delgrado et al., 1998) and reduction of cytokine levels in inflammatory exudates in vivo (Getting et al., 1999). This anti-inflammatory effect has been shown to occur via inhibition of nuclear transcription factor-kappa B (NF-κB) activation (Delgrado et al., 1998; Taherzadeh et al., 1999) and protection of IκBα degradation (Ichiyama et al., 1999). Recently a potential new mechanism of action has been postulated with discovery that melanocortin peptides can induce the anti-inflammatory protein heme-oxygenase 1 (HO-1). At least part of the anti-inflammatory effects of melanocortins is dependent on HO-1, as its blockade with a specific inhibitor (zinc protoporphyrin IX), abrogates their anti-inflammatory effects (Lam et al., 2005). These intracellular events would produce a reduction in the expression of pro-inflammatory cytokines and adhesion molecules (Scholzen et al., 2003), thereby affecting the humoral and cellular phases of inflammation (Lam and Getting, 2004). Melanocortin peptides also play an important protective role in models of ischaemic heart damage, where a role for the melanocortin MC_3 receptor has been highlighted (Getting et al., 2004). The role played by the emigrating leukocyte (predominantly neutrophils) and generation of cytokines and chemokines in both these inflammatory (Getting et al., 1997a) and cardiovascular pathologies has been demonstrated (Latini et al., 1994; Blum, 1996; Maulik et al., 1993).

In this study we have utilised the clinically effective melanocortin peptide HP228 (Weinger et al., 1998) which possesses beneficial effects in pre-clinical models of endotoxin shock by inhibiting tumour necrosis factor (TNF)-α and interleukin (IL)-1 levels (Abou-Mohamed et al., 1995) and a potential therapeutic effect in prolonged diabetic neuropathy (Calcutt et al., 1998). Given these pre- and clinical protective effects of HP228, we have utilised pre-clinical models of acute inflammation and myocardial ischaemia to ascertain its potential benefits.

2. Materials and methods

2.1. Animals

Male Swiss Albino mice (20–22 g body weight) were purchased from Bantin and Kingman (CD1 strain; Hull, Humberside) and were maintained on a standard chow pellet diet with tap water ad libitum using a 12 h light/dark cycle. Animals were used 7 days after arrival according to guidelines laid down by the ethical committee for the use of animals, Bart's and The Royal London School of Medicine and Dentistry. Animal work was performed according to Home Office regulations (Guidance on the Operation of Animals, Scientific Procedures Act, 1986).

2.2. Materials

NDP-α-MSH (Nle^4, D-Phe^7)-α-MSH, HP228 (Ac-Nle^4, Gln^5, D-Phe^7, D-Trp^9)-αMSH (4–10), MTII (Ac-Nle^4-c[Asp^5, D-Phe^7, Lys^{10}]NH_2 $ACTH_{4–10}$), SHU9119 (Ac-Nle^4-c[Asp^5, D^2-Nal^7, Lys^{10}]NH_2 $ACTH_{4–10}$) and HS014 (Ac–Cys^3–Nle^4–Arg^5, D^2–Nal^7, Cys^{11})-α-MSH–NH_2 were obtained from Bachem UK Ltd (St. Helens, Merseyside, UK). Zymosan type A, carrageenan, phosphate buffered saline (PBS), ethylene-(2,2)-diamine-tetracetic acid (EDTA), isobutylmethylxanthine (IBMX), Forskolin and all other chemicals were purchased from Sigma Chemical Co. (Poole, UK), IL-1β, TNF-α KC enzyme-linked immunosorbant assay (ELISA) kits (R and D Systems, Abingdon, Oxon UK), cAMP enzyme immunoassay (EIA) (Amersham Ltd, Little Chalfont, Buckinghamshire, UK).

2.3. Drug treatment

The melanocortin peptide HP228 (Weinger et al., 1998; Calcutt et al., 1998) was dissolved in sterile PBS and given sub-cutaneously (s.c.) in the range of 0.1–1.0 mg/kg per mouse 30 min prior to stimulus. For the carrageenan air-pouch model, HP228 (0.5 mg/kg) was dissolved in PBS and administered s.c. or i.p. either 30 min prior to stimulus and in some experiments +3 h after stimulus. Two controls were used in these studies either NDP-α-MSH (0.01 mg/kg) (Sawyer et al., 1980) or dexamethasone (1 mg/kg) both were dissolved in sterile PBS (Getting et al., 1997b), in all cases controls mice received PBS alone.

2.4. In vivo models of inflammation

2.4.1. Zymosan and MSU crystal peritonitis

The peritonitis was induced by injection of 1 mg zymosan A (Getting et al., 1997b) or 3 mg monosodium urate crystals (Getting et al., 1997a) in 0.5 ml sterile PBS. The animals were then killed at 4 and 6 h, respectively, post-challenge by CO_2 exposure, peritoneal cavities washed with 3 ml of PBS containing 3 mM EDTA and 25 μ/ml heparin. Aliquots of lavage fluid were then stained with Turk's solution (0.01% crystal violet in 3% acetic acid) and differential cell counts performed using a Neubauer haemocytometer and a light microscope (Olympus B061). Lavage fluids were then centrifuged at 400 $g \times 10$ min and supernatants stored at −20 °C prior to several biochemical determinations. Data are reported as 10^6 neutrophils per mouse calculated.

2.4.2. Carrageenan air-pouch

Air-pouches were formed on the back of mice by air injection (2.5 ml s.c.) on day 0 and day 3. On day 6, 1 mg carrageenan (type lambda; 0.1 ml of a 1% suspension in sterile