Annexin 1 and Melanocortin Peptide Therapy for Protection Against Ischaemic-Reperfusion Damage in the Heart

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Cardiovascular disease is a major cause of mortality within the western world affecting 2.7 million British people. This review highlights the beneficial effects of naturally occurring hormones and their peptides, in myocardial ischaemic-injury (MI) models, a disease pathology in which cytokines and neutrophils play a causal role. Here we discuss two distinct classes of endogenous peptides: the steroid inducible annexin 1 and the melanocortin peptides.

Annexin 1 and the melanocortins counteract the most important part of the host inflammatory response, namely, the process of leukocyte extravasation, as well as release of proinflammatory mediators. Their biological effects are mediated via the seven transmembrane G-protein-coupled receptors, the fMLP receptor family (or FPR), and the melanocortin receptors, respectively. Pharmacological analysis has demonstrated that the first 24 amino acids of the N-terminus (termed Ac2-26) are the most active region. Both exogenous annexin 1 and its peptides demonstrate cardioprotectiveness and continuing work is required to understand this annexin 1/FPR relationship fully. The melanocortin peptides are derived from a precursor molecule called the POMC protein. These peptides display potent anti-inflammatory effects in human and animal models of disease. In MI, the MC3R has been demonstrated to play an important role in mediating the protective effects of these peptides.

The potential anti-inflammatory role for endogenous peptides in cardiac disease is in its infancy. The inhibition of cell migration and release of cytokines and other soluble mediators appears to play an important role in affording protection in ischaemic injury and thus may lead to potential therapeutic targets.

KEYWORDS: annexin 1, melanocortin, neutrophil, migration, anti-inflammatory, macrophage, heart, ischaemic-reperfusion, cytokine, chemokine
MYOCARDIAL ISCHAEMIA REPERFUSION

Cardiovascular disease is the leading cause of death in people over 65 years of age. At least one-third of all cardiovascular disease is attributable to five risk factors: smoking, alcohol, high blood pressure, high cholesterol, and obesity. Indeed, it is believed that over a billion people will die from cardiovascular disease in the first half of the 21st century (Anthony Rogers, Clinical Trials Research Unit, University of Auckland, NZ, 2004). The financial costs of treating such people are rapidly increasing and, therefore, a greater understanding of the disease should lead to more effective treatments, a reduction in finance, and an increase in “peace of mind”.

Ischaemia reperfusion (IR) is implicated in many different cardiac conditions, including thrombolysis, angioplasty, and coronary bypass surgery, which are frequently used to establish the blood reflow and minimise the damage to the heart due to severe myocardial infarction. In a clinical situation of myocardial IR (MIR), surgeons are able to intervene at certain points, such as at the time of coronary catheterisation or entry into emergency care facility[1].

Early induction of reperfusion is an absolute prerequisite for the survival of ischaemic myocardium[2] and can be an effective way to prevent the progression of ischaemic cell necrosis. However, reperfusion is a double-edged sword, and the restoration of blood flow into a previously ischaemic zone is not always totally beneficial. Although a significant amount of injury occurs due to ischaemia itself, a great deal of damage also occurs to the myocardium because of reperfusion.

During myocardial reperfusion, several mechanisms mediate vascular injury. The production of oxygen free radicals (OFRs) is increased by a number of different factors, such as (1) mitochondrial respiration (ischaemia changes the redox state and promotes xanthine), (2) an increase in activated neutrophils, and (3) up-regualtion of xanthine oxidase activity. This activates leukocytes, induces lipid peroxidation, and increases vascular permeability[3]. Several models of MIR have shown there to be a sudden efflux of oxygen metabolites (e.g., superoxide radical, hydroxyl radical, and peroxynitrite) when oxygen is allowed back into a previous ischaemic area[4]. These oxygen metabolites are extremely reactive and cause irreversible damage to cell membranes; the addition of superoxide dismutase reduces OFR concentrations in reperfused myocardium[5]. In ischaemic-reperfused hearts, depression of contractile function, arrhythmias, change in gene expression, and loss of adrenergic pathways have also been observed, partly due to increased OFRs. It still remains controversial whether OFRs increase during ischaemia, although it is accepted that their formation occurs during reperfusion. There is also an activation of complement[6], decreases in nitric oxide production[7], and increases in leukocyte-endothelial cell interactions, which can cause capillary plugging and the “no-reflow” phenomena[3]. Some studies have shown that active capillary plugging, i.e., the ability of pericytes (distributed along the capillaries) to induce constriction in these capillaries by contracting cell processes that partially envelope the capillary[8], may cause the reduction in cardiac capillary cross-sectional dimensions in IR injury. This idea still remains controversial, but it does appear that the capillary bed may play a much greater role in the local control of blood flow than was once previously thought.

Although there is a reduction in infarct size when neutrophils are depleted using antineutrophil antiserum[9] or leukopak filters[10], the specific role of neutrophils still remains unclear. An abundance of experimental evidence points to an important role of the selectins and adhesion molecules in neutrophil recruitment during inflammation. P-selectin antibodies, e.g., PB1.3, have been shown to reduce infarct size and associated risk, along with attenuating endothelial dysfunction[11,12]. Also, a recombinant analogue of the major PSGL-1 has been shown to be protective in a feline model of coronary occlusion and reperfusion[13]. L-selectin monoclonal antibody also reduces myocardial necrosis by approximately 50% in a feline model[14]. E-selectin KO mice also have smaller infarct sizes post-IR[15]. The blockade of ICAM-1[16] or CD18[17] partially attenuates myocardial reperfusion injury. However, it is clear that myocardial injury in-vivo is not dependent on any one adhesion molecule.

Indeed inflammation is now thought to play a serious role in both the initiation and progression of cardiovascular disease. Many different animal studies and clinical trials have investigated the risk factors associated with cardiovascular disease, such as obesity, hypertension, and smoking, and have provided
clear evidence to suggest that various aspects of the inflammatory response associated with these risk factors do heighten the damaging effect. Much evidence has been provided by the investigation of the endothelium, as endothelial dysfunction is an early event and expression of the adhesion glycoproteins by activated endothelial cells is a rate-limiting step in the recruitment of inflammatory cells\[18\].

However, despite the growing body of evidence that inflammation plays a key role in the progression of cardiovascular disease, many clinical trials have failed, e.g., to date, no antineutrophil clinical trials have been successful. Trials using humanised anti-CD18 mAbs (FESTIVAL\[19\]) and LIMIT-AMI\[20\] have been administered to patients with myocardial infarction, but no effect was observed in infarct size.

**CURRENT THERAPIES**

Table 1 demonstrates the vast choice of medication that is available to treat and manage cardiovascular disease.

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**TABLE 1**

*Medication for the Treatment and Management of Cardiovascular Disease (Adapted from World Health Organisation)*

| Medication Type                          | Used For                                                                 | Examples  |
|-----------------------------------------|--------------------------------------------------------------------------|-----------|
| Antiplatelet agent                      | Prevention of blood clots                                                | Statins   |
| Anticoagulant or blood thinner          | Prevention of blood clots. Used in patients with atrial fibrillation and after heart valve replacement surgery. | Warfarin  |
| Vasodilator                             | Blood vessel relaxation. Prevention and relief of angina.                | Nitrates  |
| Diuretic                                | Removes excess water from the body and prevents build up. Lowers blood pressure. Used for high blood pressure and heart failure. | Furosemide, Thiazides |
| Calcium-channel blockers                | Relaxes blood vessels and lowers blood pressure. Used for high blood pressure and angina. | Nifedipine |
| Beta-blocker                            | Slows the heart rate and increases force of heartbeat. Used for high blood pressure and angina. | Atenolol  |
| Angiotensin-converting enzyme (ACE) inhibitor | Relaxes blood vessels and reduces strain on the heart. Used for high blood pressure. | Enalapril |
| Centrally acting antihypertensive       | Lowers blood pressure by acting on the brain.                           | Methylodopa |
| Angiotensin II receptor blocker (ARB)   | Dilates blood vessels and lowers blood pressure.                         | Cadesartan |
| Cardiac glycoside                       | Increases the strength of heart muscles and helps heart pump blood. Used for heart failure. | Digoxin   |
| Blood cholesterol–lowering agent        | Lowers cholesterol level.                                                | Statins   |
| Biguanide                               | Helps body cells to take in sugar. Used for diabetes.                    | Metformin |
| Sulfonylurease                          | Increases insulin production. Used for diabetes.                         | Glibencamide |
However, there are several lines of approach that are currently being investigated for the treatment and management of cardiovascular disease. Some of these therapies are as follows:

1. Creation of new drugs that increase the levels of high-density lipoproteins (HDLs), which in-turn will lower heart disease.
2. Development of angiogenesis drugs, which will aid the growth of new arteries on the heart, and thus reduce the need for bypass surgery.
3. Develop antiglycosylation therapies for the prevention of cross-linking that weakens aging heart muscle, and reduce diabetic risk factor.
4. Investigation of combining nicotinic acid (a potent agent for increasing HDL cholesterol and reducing LDL cholesterol) with a statin, which may help to reduce coronary heart disease[21].
5. Gene therapy as a potential strategy for treating cardiovascular disease; combining this therapy with cell therapy and tissue engineering, gene silencing, and the targeting of genes in the vascular wall and the myocardium.

Another line of interest is the study of inflammatory markers and mediators that may lead to potential therapeutic strategies and drug targets, with the development of compounds that may have fewer side effects. The potential anti-inflammatory role for endogenous peptides in cardiac disease is in its infancy. The inhibition of cell migration and release of cytokines and other soluble mediators appears to play an important role in affording protection in ischaemic injury and thus may lead to potential therapeutic targets. This review will focus on the beneficial effects of two different types of endogenous peptides: annexin 1 and melanocortin.

ANNEXIN 1

Glucocorticoids are synthesised by the adrenal cortex and endogenously released to reduce the inflammatory response, e.g., they can act as potent anti-inflammatory drugs by inhibiting phospholipase A2 (PLA2) activity, as well as cyclooxygenase-2 (COX-2) and inducible nitric oxide species (iNOS) expression, thus preventing autocoid release and function[22]. Glucocorticoids are also potent inhibitors of neutrophil tissue damage[23].

In the late 1970s, a new protein was discovered and characterised by its ability to quash inflammatory mediators of the eicosanoid family by suppressing the activity of the enzyme PLA2. The action on arachidonate and eicosanoid release in vitro[24] was accompanied by an inhibitory effect in experimental models of inflammation in vivo (e.g., TXA2 release from perfused guinea pig lungs[25]. This novel protein is now termed “annexin 1”.

Annexin 1 is a 37-kDa (consisting of 346 amino acids; Fig. 1) member of a superfamily of proteins (there are 13 mammalian annexins). The larger portion of this protein is formed by a 70-amino-acid motif, which is repeated four times, which are then grouped together to form a globular structure with a convex and concave face. Binding of Ca2+ and phospholipids to annexin is mediated through a type II and type III Ca2+-binding site within the core domain, and Ca2+ bound within these sites serves as a platform through which they interact with peripheral membrane phospholipids. A unique N-terminus attached to this core domain varies in length between the different members of the superfamily (Fig. 1).

In murine peripheral leukocytes, annexin 1 concentrations are highest in neutrophils and lowest in lymphocytes, with an intermediate amount in monocytes[26]. The glucocorticoid dexamethasone (DEX) increased annexin 1 expression in neutrophils and monocytes. In human leukocytes, annexin 1 is found in largest amounts in neutrophils and monocytes, with low and varying amounts in T-lymphocytes[27], and no expression in B lymphocytes. The largest pool in lymphocytes is in CD56+ natural killer cells[28].
FIGURE 1. A schematic representation of annexin 1 and its N-terminal domain. The four repeats of annexin 1 are shown in which repeat 1 and 4 and repeat 2 and 3 are paired. The N-terminal domain is attached to repeat 1.

Since annexin 1 is most abundant in neutrophils, it is considered to be the most likely candidate to mediate calcium-dependent membrane fusion during phagocytosis and/or exocytosis of neutrophils. When neutrophils adhere to endothelial cells, annexin 1 is externalised from its storage site (gelatinase granules) to the cell surface[29] where the endogenous protein acts in an auto/paracrine manner to inhibit the process of leukocyte diapedesis. This reduction in inflammation by annexin 1 is achieved in different ways, such as reduced paw oedema, decreased PMN migration, antipyretic effects, and an antiendotoxic action. In vitro, this effect is observed when neutrophils adhere to monolayers of endothelial cells, annexin 1 is externalised onto the cell surface, down-regulating the cell’s emigration and thus activating other secondary pathways.

Following migration into the tissue, annexin 1 is cleaved. Perretti al.[30] used anti-annexin 1 sheep serum, raised against the full length of annexin 1 to investigate this aspect. Intravascular neutrophils adherent to the endothelium retained cell surface annexin 1 in its intact form (i.e., 37 kDa), whereas annexin 1 in migrated cells was mostly cleaved (i.e., 34 kDa) and localised in large endosome-like vacuoles. This study suggests that the immunoreactivity of the vacuoles of extravasated neutrophils is due to internalised intact annexin 1. Western blotting analysis also shows annexin 1 cleavage during inflammation. A “lipocortinase” has been proposed to be responsible for this cleavage, being selectively activated during the process of neutrophil adhesion/emigration[31].

Annexin 1 and Its Peptides

Peptides (Fig. 2) derived from the N-terminus of annexin 1, e.g., Ac2-26, Ac2-12, and Ac2-6, have been used in many studies[32]. The peptide spanning the first 24 amino acids of annexin 1, termed Ac2-26, has been shown to mimic the human recombinant annexin 1 for its ability to inhibit neutrophil migration in inflammatory models. Peptide Ac2-26 is approximately 200 times less potent than the parent compound, although the dose-response curve is parallel, implying similar efficacy[33]. The technique of intravital microscopy has been used to demonstrate that both annexin 1 and peptide Ac2-26 are able to inhibit blood-borne leukocyte extravasation. To be more specific, annexin 1 and its mimetic peptide demonstrated that only the fate of adherent leukocytes was affected, whereas rolling and the extent of leukocyte adhesion were unaffected[34,35].
Other peptide sequences have been derived from the core of annexin 1; these are termed the antiflammins[36] (Fig. 2). One encompasses amino acids 39-47 (MQMKKVLDS) of an uteroglobin sequence and is termed antiflammin 1 (AF-1), and a second from the 246-254 (HDMNKVLDL) of annexin 1, termed antiflammin 2 (AF-2). The antiflammins also inhibit cytosolic phospholipase A₂ (cPLA₂) in a concentration-dependent manner in vitro, and reduce oedema formation following local injection of carrageenin into the rat paw[36]. However, some authors have found it difficult to repeat the inhibitory effect on cPLA₂ activity[37,38], raising doubts about the real effectiveness of the antiflammins as enzyme inhibitors. Nonetheless, antiflammins have been shown to inhibit leukocyte adhesion to human leukocytes and coronary artery endothelial cells, by attenuating activation-induced upregulation of CD11/CD18 on leukocytes[39].

**Annexin 1 and Its Receptors**

In 2000, Walther and colleagues confirmed, using *in vitro* techniques, the antimigratory effects of peptide Ac2-26, and linked Ac2-26 to the receptor for formylated peptide (FPR)[40]. This group showed that Ac2-26 provoked transient changes in intracellular calcium and L-selectin shedding from the plasma membrane, which were prevented by the addition of FPR antagonists, i.e., the butoxyl-carbonyl (or Boc) derivatives.

The FPR is a G-protein-coupled receptor found mainly on leukocytes. It is one of the best-studied chemoattractant receptors and, like all other chemoattractant receptors, FPR produces a range of
responses on stimulation, e.g., stimulation of migration and, at higher doses, opening calcium channels, triggering with exocytosis and a respiratory burst[41].

Two human genes that encode specific FPR subtypes have been cloned; they are termed FPR1 and FPRL1 (FPR-like 1, also referred to as FPRH2, and a functional lipoxin A₄ [LXA₄] receptor or ALXR). Both are present on human neutrophils and monocytes, and FPRL1 is also present on epithelial cells[42]. FPR binds fMLP with approximately 1000-fold higher affinity than FPRL1, resulting in calcium mobilisation and subsequent neutrophil activation. FPRL1 shares approximately 69% amino acid identity with FPR, particularly in the signalling domain and thus it has been suggested that FPR and FPRL1 are likely to transduce the same signal downstream of the receptor, but to be activated by different ligands. Various agonists that preferentially bind and activate FPRL1 have been identified, e.g., the HIV-derived peptide, V3[43]. FPRL1 also transduces anti-inflammatory signals, as is the case with LXA₄. Construction and screening of random peptide libraries is a useful approach to developing biologically active agents. Klein et al. isolated a number of small peptide sequences that reacted with FPR and FPRL1[44], e.g., MMK-1 (LESIFRSLLFRVM), which is one of the most potent FPRL1-specific agonists identified so far. FPRL2 has been identified by cross-hybridisation, but as of yet its function remains unclear[45].

**Murine FPR Family**

Having described the human FPR family, the plot thickens considerably in rodents[46]. Currently, there appear to be eight structurally related genes: *Fpr, Fpr-rs1, Fpr-rs2, Fpr-rs3, Fpr-rs4, Fpr-rs5*, *Fpr-rs6, Fpr-rs7*[48], and possible three distinct proteins[47]. *Fpr* is the orthologue of human FPR and Gao et al. suggested that the human FPRL1 gene consists of both *Fpr-rs1* (which appears to be the murine receptor for LXA₄[49]) and *Fpr-rs2* (which is similar to human FPRL1 and FPRL2[50] in the mouse). Wang and colleagues also reported the presence of another murine receptor for LXA₄[48]. Mouse *FPR* displays a significantly lower affinity for its agonist, fMLP, such that micromolar concentrations of this formulated tripeptide are required to activate it[50].

The above suggests that the water is muddy for mouse FPRs. It is not clear whether this complex system is purely due to a problem in nomenclature by the various groups working in the field. Also, as of yet, not all these genes appear to be expressed as proteins and it remains to be seen whether this will happen. Species similarities are now being noted, e.g., a rat LXA₄ receptor has been cloned[51], which shares 74 and 84% amino acid homology with human and mouse orthologues.

**Annexin 1 and MIR**

Annexin 1 has been shown to be cardioprotective in both rat and mouse MIR models[32,34,52]. Indeed this cardioprotective action displayed by annexin 1 and its N-terminus–derived peptides appears to be specific, as the structurally related annexin 5 did not reduce ischaemic damage in a rat model[32]. The expression of endogenous annexin 1 in this MIR model demonstrated that hearts from sham or naïve animals did not express annexin 1, as expected[53]. However, as seen in other inflammatory conditions[54,55], tissue infiltration brings about annexin 1 expression. In IR treated hearts, annexin 1 appeared as a characteristic doublet, with bands being found at 34 and 37 kDa. When rats were treated with Ac2-26, the doublet appeared reduced: the 37-kDa band being stronger than 34 kDa.

The enzyme responsible for this catabolism of annexin 1 is unclear, but the 34-kDa fragment lacks anti-inflammatory activity[56]. It may be that peptide Ac2-26 may compete with the intact form of annexin 1 at the enzyme level, thus reducing endogenous annexin 1 catabolism, which may help to explain why Ac2-26 displays cardioprotective action. It is also true, though, that peptide Ac2-26 may reduce the total amount of annexin 1 by reducing leukocyte extravasation, thereby indirectly reducing the extent of protein degradation detected in the inflamed tissue.
A similar effect was observed in the murine MIR model[34], in which peptide Ac2-26 was able to inhibit the effect of IR damage. By using FPR null mice, it was possible for the first time to investigate this effect further and demonstrate that the cardioprotective effect was due to an involvement of a Boc2 sensitive receptor other than the FPR.

Other members of the annexin superfamily have been investigated in relation to cardiovascular implications. During contractile dysfunction in congestive heart failure, altered levels of intracellular calcium ions have been found[57]. The annexins are a family of Ca\(^{2+}\)-binding proteins that are abundant in the heart (but not annexin 1) and thus may play a role in cardiac excitation-contraction coupling (which is often used to elucidate the intracellular mechanisms associated with contractile dysfunction in congestive heart failure). Matteo et al. demonstrated that alterations in the intracellular localisation of annexins (focussing on annexins 4, 5, and 6), along with up-regulation of annexins 5 and 6 in failing heart cells, suggested differential regulation of these annexins[58]. No data were provided about annexin 1, so it is not clear as to whether this particular Ca\(^{2+}\) regulatory protein is involved in cardiac excitation-contraction coupling. However, some evidence suggests that the intracellular mechanism of the actions of annexin 1 may be linked to alterations in Ca\(^{2+}\) handling, probably at the level of release from cytosolic stores rather than influx into the cell[59,60], similar to the actions of the glucocorticoid DEX[61].

**MELANOCORTINS**

Melanocortins are ancient peptides little changed throughout evolution[62] and were first identified in common ancestors of lampreys and gnathostomes 700 million years ago[63]. These peptides are derived from a larger precursor molecule known as the pro-opiomelanocortin (POMC) protein and have been detected in the hypothalamus, pituitary, and periphery (including the immune system, spleen, lung, melanocytes, and the gastrointestinal tract[64]). The melanocortins all share a common amino acid sequence, His-Phe-Arg-Trp (HFRW), a sequence required for receptor binding, activation, and mediating their biological effects. The POMC protein contains three main domains: the N-terminus region, which contains \(\gamma\)-MSH, the central highly conserved ACTH\(_{1-39}\) sequence, with \(\alpha\)-MSH at its N-terminus, and the C-terminal \(\beta\)-lipotropin, which can be cleaved to generate \(\beta\)-endorphin[65]. The POMC protein is cleaved into its biologically active products following proteolytic cleavage by prohormone converting (PC) enzyme and carboxypeptidases between two pairs of basic amino acid residues (-Lys-Lys, -Arg-Lys-, -Arg-Arg-, -Lys-Arg-). There are seven members of the PC family, including PC1/3, PC2, furin/PACE, PACE4, PC4, PC5/6, and PC7/SPC7/LPC/PC8[66], with PC1 cleaving POMC protein into ACTH\(_{1-39}\) and \(\beta\)-lipotropin together with low concentrations of \(\beta\)-endorphin, whilst PC2 cleaves POMC protein into \(\beta\)-endorphin and \(\beta\)-MSH[67].

**Melanocortin Receptors**

Melanocortins exert their biological effects by binding to G-protein-coupled seven transmembrane receptors (GPCRs). They are the smallest family of GPCRs due to the fact that they have a small second extracellular loop and short amino and carboxy terminal ends[68]. Five melanocortin receptors (MCR) have been cloned and termed MC1R to MC5R; they are positively coupled to adenylylate cyclase with agonism leading to cAMP accumulation within the target cell. They have been shown to have a high sequence homology and can be detected in many different tissues[68].

- **MC1R** — This receptor displays many different functions, including pigmentation and antipyretic and anti-inflammatory actions. Expression occurs in a number of tissues including melanocytes, RAW264.7[69] and THP-1 macrophage (MØ) cell lines[70], human monocytes[71], neutrophils[72], endothelial cells[73], fibroblasts[74], mast cells[75], and
lymphocytes[76]. Although predominately a peripheral receptor, it has been detected in rat and human brains in neurons of the periaqueductal gray substance[77].

- **MC2R** — The MC2R is unique since only ACTH_{1-39} will activate this receptor with no biological efficacy with the melanocortin peptides. Activation of the receptor leads to a regulation in the release of steroids by the adrenal cortex essential for promotion of steroidgenic enzymes[78]. Although it is only thought to be expressed on the adrenal gland, expression has also been detected on adipocytes of mice[79], but not human[80]. Given this expression, there is the possibility that a role in metabolism might exist.

- **MC3R** — Expression occurs in the central nervous system, peripheral tissues, and immune cells, with initial studies highlighting expression in brain, gut, and placenta, but no detection in the adrenal gland or melanocytes[81]. MC3R has been postulated to be involved in modulating energy metabolism, since in MC3R null mice there is an increased fat mass and higher ratio of weight gain to food intake[82]. A role in modulating the host inflammatory response has been proposed with identification of message and protein for the receptor on peritoneal[83,84,85] and knee joint MØ[86]. Activation of resident MØ has been shown to lead to an initial inhibition of proinflammatory cytokines and chemokines, whilst at later time-points, the induction of anti-inflammatory cytokines and proteins, such as heme-oxygenase 1[87]. Finally, a role in mediating the protective effects of the melanocortins in IR injury exists[88,89]. Thus, MC3R could be proposed as a fine tuner of specific mechanisms operating during inflammation, cardiovascular function, and energy metabolism.

- **MC4R** — This receptor is unique given that it is solely expressed within many regions of the brain, including the hypothalamus, spinal cord, and cortex[90]. Most interest from the pharmaceutical industry has focused on its role in controlling food intake and energy expenditure, and could be an exciting target for controlling obesity. Other potential applications are in modulating erectile dysfunction[91] and pain[92].

- **MC5R** — Like the MC2R, MC5R is solely found in the periphery being detected in liver, lung, thymus, testis, ovary, mammary glands, fat cells, bone marrow, skin, skeletal muscle, stomach, and duodenum[68]. It is also expressed in B[93] and T[94] lymphocytes, suggesting a role in immune regulation.

### Melanocortins and MIR

Targeting of inflammatory processes, such as inhibiting proinflammatory cytokines and neutrophil migration, could be beneficial in treating this disease pathology, given the causal role that leukocytes and cytokines play in myocardial ischaemia (MI). Melanocortins are potent anti-inflammatory agents inhibiting leukocyte migration and release, and actions of cytokines and chemokines[95], therefore, they have been investigated in this pathology. Both murine and rat models of MI have been used to demonstrate the protective effects of the melanocortins. Utilising a rat model of short-term ischaemia (5 min), caused by ligation of the left anterior descending coronary artery, the parent hormone ACTH_{1-24} and the potent nonselective agonist NDP-α-MSH were effective in significantly reducing incidences of arrhythmias, lethality, and free radicals in the blood[96].

Following these initial observations, pharmacological, genetic, and molecular approaches have been undertaken to identify the receptor(s) involved in mediating this protection. To rule out a role for the MC2R expressed on the adrenal gland, a surgical approach was undertaken with rats being adrenalectomised. Following this, the protective effects of the melanocortins were still evident, indicating a potential role for other melanocortin receptors.

To try to dissect the role played by individual receptors, a panel of pharmacological tools have been used, including the selective MC1R agonist MS05[97] and selective antagonists directed at the MC4R (HS014)[98] and MC5R (HS059)[88]. MS05 failed to exert a protective effect in this model, whilst the antagonists did not attenuate the protective actions of ACTH_{1-24}. These data would indicate that a role for
the MC1R, MC4R, and the MC5R could be excluded. Given these findings, the potential role for MC3R was evaluated using the MC3/4R antagonist SHU9119, which attenuated the protective effect, suggesting an involvement of MC3R in affording protection within the heart[88]. To try to dissect the role played by the MC3R and to confirm its pivotal role in affording protection, further pharmacological manipulation with compounds that show a selectivity towards MC3R have been utilised. Protective effects were observed with $\gamma_2$-MSH[84,86] and [D-TRP$^8$]$\gamma_2$-MSH[99], which reduced the incidence of ventricular tachycardia, fibrillation, and death, as well as an increase in free radical blood levels and fall in arterial pressure[100]. All these studies highlighted a role for MC3R utilising pharmacological rather than genetic or molecular approaches.

Recently, the cellular target for the MC3R within the heart and also the effect of the MC3/4R agonist MTII in a more chronic model of IR injury have been evaluated looking at 24-h postreperfusion. RT-PCR and western blotting highlighted message and protein for the MC3R in mouse and rat hearts; this expression was unaltered following IR. Electron microscopy showed immunogold labelling of MC3R on heart MØ, but not fibroblasts or cardiomyocytes with a punctuate distribution of receptors on the cell surface; the identification of the molecular target was further substantiated in vivo. Administration of the MC3/4R agonist MTII attenuated mouse heart 2-h reperfusion injury by $\sim$40%, an effect prevented by the mixed MC3/4R antagonist SHU9119, but not by the selective MC4R antagonist HS024. To rule out a role for the MC1R, the recessive yellow (e/e) mouse, bearing a mutated (inactive) MC1-R[101], was used with MTII, displaying a fully protective effect. Given the protective effects of melanocortin peptides in MIR, biochemical markers of inflammation have been monitored to see if they are modified by MTII. MTII reduced markers of systemic and local inflammation, including cytokine contents (interleukin-1 and KC) and myeloperoxidase activity. MTII has more importantly been shown to be effective when given at the beginning of the reperfusion period and after delayed myocardial damage as measured 24-h postreperfusion, thus suggesting a beneficial effect in acute and delayed heart reperfusion injury. These studies highlight a previously unrecognised protective role for MC3R activation and may open up new avenues for therapeutic intervention against heart and possibly other organ IR injury[89]. Table 2 summarises these findings.

### TABLE 2
Melanocortin Peptides Effects in Myocardial-Reperfusion Injury

| Peptide Treatment | Observed Effects                                                                 | References |
|-------------------|----------------------------------------------------------------------------------|------------|
| ACTH$_{1-24}$     | Reduction in incidence of arrhythmias and lethality. Total abrogation in ischaemia-reperfusion injury induced free radicals. | [96]       |
|                   | Reduced ventricular tachycardia, fibrillation, and lethality. Protective effects not blocked by HS014 or HS059, but abrogated by SHU9119. | [88]       |
| NDP-$\alpha$-MSH   | Reduction in incidence of arrhythmias and lethality. Total abrogation in ischaemia-reperfusion injury induced free radicals. | [96]       |
| MS05              | Unable to prevent damage                                                          | [88]       |
| $\gamma_2$-MSH     | Reduction in incidence of ventricular tachycardia, fibrillation, and lethality    | [100]      |
| [D-TRP$^8$]$\gamma_2$-MSH | Reduced ischaemia at 2 and 24 h, effective when given at start of reperfusion. MC3R mRNA and protein detected in heart MØ. Inhibition of IL-1, KC, and MPO levels. All parameters inhibited in recessive yellow e/e mice. Protective effects blocked by SHU9119, but not by HS024. | [89]       |

MTII
FIGURE 3. Schematic representation of the anti-inflammatory effects of the annexin and melanocortin peptides. Annexin 1 causes detachment of neutrophils from the endothelium by being released from gelatinase granules to the surface on activation of the cell. The detachment process could occur via two mechanisms: (1) the full-length protein could interact with receptor(s) to down-regulate neutrophil migration or (2) the protein is cleaved to make smaller pharmacologically active peptides that can also bind to the receptor and prevent neutrophil migration and also effect L-selectin shedding (highlighted in the smaller cartoon). The melanocortins display a different mechanism for modulating the host inflammatory response, resident MØ express MC3-R and agonism here leads to accumulation of cAMP with consequent activation of PKA. At least two major effects occur, with an early inhibition of release of proinflammatory cytokines/chemokines within the first 4 h and at later time-points, >4-6 h, the induction of the stress protein HO-1. Either mechanism of action can impact on the host inflammatory response and be responsible for the potent and reproducible antimigratory effects that melanocortin peptides exert in the context of acute local and systemic inflammation. The net effect with these endogenous peptides is that they modulate the host inflammatory response leading to a homeostatic balance within the tissue.
CONCLUDING COMMENTS

The generation of proinflammatory cytokines and the associated inflammatory response are part of protective mechanisms initiated by the host to counteract insults and foreign pathogens, and reinstall tissue and organ homeostasis. Sometimes, though, the inflammatory process is more detrimental than life saving, as in the case of chronic inflammatory pathologies, including rheumatoid arthritis and inflammatory bowel disease[102]. This can be particularly true also for the tissue injury that follows IR of the heart[6,103]. The inflammatory response that ensues has the ultimate scope of resolving the damage, however it predisposes to tissue remodelling[15,104,105]. Targeting endogenous modulators of inflammation such as annexin 1 and the melanocortins could be an exciting avenue for future drug discovery. These peptides display distinct mechanisms of action, although both exert a protective effect. Annexin 1 prevents the circulating neutrophil from adhering to the endothelium and thus inhibiting its migration into tissue, whilst the melanocortins prevent activation of the endothelium by switching off the production of proinflammatory mediators from resident cells.

These compounds might have a potential advantage over existing therapies in that they may dampen down the host’s response to inflammation, infection, and ischaemia, and play a protective role in maintaining a homeostatic balance within the body. Given their multitudes of action, in some respects they act like a “sledgehammer”; like a steroid, but without the side effects. It is always difficult to postulate whether peptide molecules could be first-line therapeutics due to the rapid clearance and moderately short half-lives. Although this may be a potential problem, they will have the advantage in the fact that they will not accumulate and some of the side effects associated with conventional treatments may be avoided[68]. In the future, new receptors, more potent and longer-lasting derivatives may be discovered. Another exciting opportunity may be in the development of dual inhibitors, such that the annexin 1 portion could be used to inhibit the rolling stage of the leukocyte, whilst the melanocortin portion will prevent the endothelium from becoming sticky. The net result would be inhibition of leukocyte migration to the site of tissue injury. Based on this, the potential use of lower doses of compounds might exist and, therefore, reduction of potential side effects might occur. What is clear is that we are entering an era where promoting the body’s own natural defences could lead to novel therapeutics. Fig. 3 highlights the mechanism of action for these endogenous anti-inflammatory peptides.

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REFERENCES

1. Jordan, J.E., Zhao, Z.Q., and Vinten-Johansen, J. (1999) The role of neutrophils in myocardial ischemia-reperfusion injury. Cardiovasc. Res. 43, 860–878.
2. Moens, A.L., Claeys, M.J., Timmermans, J.P., and Vrints, C.J. (2005) Myocardial ischemia/reperfusion-injury, a clinical view on a complex pathophysiological process. Int. J. Cardiol. 100, 179–190.
3. Ambrosio, G. and Tritto, I. (1999) Reperfusion injury: experimental evidence and clinical implications. Am. Heart J. 138, S69–75.
4. Garlick, P.B., Davies, M.J., Hearse, D.J., and Slater, T.F. (1987) Direct detection of free radicals in the reperfused rat heart using electron spin resonance spectroscopy. Circ. Res. 61, 757–760.
5. Zweier, J.L. (1988) Measurement of superoxide-derived free radicals in the reperfused heart. Evidence for a free radical mechanism of reperfusion injury. J. Biol. Chem. 263, 1353–1357.
6. Frangogiannis, N.G., Smith, C.W., and Entman, M.L. (2002) The inflammatory response in myocardial infarction. Cardiovasc. Res. 53, 31–47.
7. Carden, D.L. and Granger, D.N. (2000) Pathophysiology of ischaemia-reperfusion injury. J. Pathol. 190, 255–266.
8. Glyn, M.C. and Ward, B.J. (2000) Contraction in cardiac endothelial cells contributes to changes in capillary dimensions following ischaemia and reperfusion. *Cardiovasc. Res.* **48**, 346–356.
9. Romson, J.L., Hook, B.G., Kunkel, S.L., Abrams, G.D., Schork, M.A., and Lucchesi, B.R. (1983) Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. *Circulation* **67**, 1016–1023.
10. Litt, M.R., Jeremy, R.W., Weisman, H.F., Winkelstein, J.A., and Becker, L.C. (1989) Neutrophil depletion limited to reperfusion reduces myocardial infarct size after 90 minutes of ischemia. Evidence for neutrophil-mediated reperfusion injury. *Circulation* **80**, 1816–1827.
11. Weyrich, A.S., Ma, X.Y., Lefer, D.J., Albertine, K.H., and Lefer, A.M. (1993) In vivo neutralization of P-selectin protects feline heart and endothelium in myocardial ischemia and reperfusion injury. *J. Clin. Invest.* **91**, 2620–2629.
12. Lefer, D.J., Flynn, D.M., and Buda, A.J. (1996) Effects of a monoclonal antibody directed against P-selectin after myocardial ischemia and reperfusion. *Am. J. Physiol.* **270**, H88–98.
13. Eppihimer, M.J. and Schaub, R.G. (2000) P-Selectin-dependent inhibition of thrombosis during venous stasis. *Arterioscler. Thromb. Vasc. Biol.* **20**, 2483–2488.
14. Ma, X.L., Weyrich, A.S., Lefer, D.J., and Lefer, A.M. (1993) Diminished basal nitric oxide release after myocardial ischemia and reperfusion promotes neutrophil adherence to coronary endothelium. *Circ. Res.* **72**, 403–412.
15. Jones, S.P., Trocha, S.D., Strange, M.B., Granger, D.N., Kevil, C.G., Bullard, D.C., and Lefer, D.J. (2000) Leukocyte and endothelial cell adhesion molecules in a chronic murine model of myocardial reperfusion injury. *Am. J. Physiol. Heart Circ. Physiol.* **279**, H2196–2201.
16. Hartman, J.C., Anderson, D.C., Wiltse, A.L., Lane, C.L., Rosenbloom, C.L., Manning, A.M., Humphrey, W.R., Wall, T.M., and Shebuski, R.J. (1995) Protection of ischemic/reperfused canine myocardium by CL18/6, a monoclonal antibody to adhesion molecule ICAM-1. *Cardiovasc. Res.* **30**, 47–54.
17. Lefer, D.J., Shandelya, S.M., Serrano, C.V., Jr., Becker, L.C., Kuppusamy, P., and Zweier, J.L. (1993) Cardioprotective actions of a monoclonal antibody against CD-18 in myocardial ischemia-reperfusion injury. *Circulation* **88**, 1779–1787.
18. Granger, D.N. and Kubes, P. (1994) The microcirculation and inflammation: modulation of leukocyte-endothelial cell adhesion. *J. Leukoc. Biol.* **55**, 662–675.
19. Rusnak, J.M., Kopecky, S.L., Clements, I.P., Gibbons, R.J., Holland, A.E., Peterman, H.S., Martin, J.S., Saoud, J.B., Feldman, R.L., Breisblatt, W.M., Simons, M., Gessler, C.J., Jr., and Yu, A.S. (2001) An anti-CD11/CD18 monoclonal antibody in patients with acute myocardial infarction having percutaneous transluminal coronary angioplasty (the FESTIVAL study). *Am. J. Cardiol.* **88**, 482–487.
20. Baran, K.W., Nguyen, M., McKendall, G.R., Lambrew, C.T., Dykstra, G., Palmeri, S.T., Gibbons, R.J., Borzak, S., Sobel, B.E., Gourlay, S.G., Rundle, A.C., Gibson, C.M., and Barron, H.V. (2001) Double-blind, randomized trial of an anti-CD18 antibody in conjunction with recombinant tissue plasminogen activator for acute myocardial infarction: limitation of myocardial infarction following thrombolysis in acute myocardial infarction (LIMIT AMI) study. *Circulation* **104**, 2778–2783.
21. Chapman, M.J. (2005) Beyond the statins: new therapeutic perspectives in cardiovascular disease prevention. *Cardiovasc. Drugs Ther.* **19**, 135–139.
22. Flower, R.J. (1988) Eleventh Gaddum memorial lecture. Lipocortin and the mechanism of action of the glucocorticoids. *Br. J. Pharmacol.* **94**, 987–1015.
23. Perretti, M., Chiang, N., La, M., Fierro, I.M., Marullo, S., Getting, S.J., Solito, E., and Serhan, C.N. (2002) Endogenous lipid- and peptide-derived anti-inflammatory pathways generated with glucocorticoid and aspirin treatment activate the lipoxin A4 receptor. *Nat. Med.* **8**, 1296–1302.
24. Parente, L., Di Rosea, M., Flower, R.J., Meli, R., Persico, P., Salmon, J.A., and Wood, J.N. (1984) Relationship between the anti-phospholipase and anti-inflammatory effects of glucocorticoid-induced proteins. *Eur. J. Pharmacol.* **99**, 233–239.
25. Cirino, G., Peers, S.H., Flower, R.J., Browning, J.L., and Pepinsky, R.B. (1989) Human recombinant lipocortin 1 has acute local anti-inflammatory properties in the rat paw edema test. *Proc. Natl. Acad. Sci. U. S. A.* **86**, 3428–3432.
26. Perretti, M. and Flower, R.J. (1996) Measurement of lipocortin 1 levels in murine peripheral blood leukocytes by flow cytometry: modulation by glucocorticoids and inflammation. *Br. J. Pharmacol.* **118**, 605–610.
27. Kim, H.W., Choi, E., Yoo, B., Choi, J.R., Park, Y.M., Lee, S.O., Moon, H.B., and Na, D.S. (1996) Lipocortin 1 binding sites on human T-cells: the population of cells with the binding sites is larger in CD8+ T-lymphocytes than in CD4+ T-lymphocytes. *Biochem. Mol. Biol. Int.* **40**, 1167–1173.
28. Morand, E.F., Hutchinson, P., Hargreaves, A., Goulding, N.J., Boyce, N.W., and Holdsworth, S.R. (1995) Detection of intracellular lipocortin 1 in human leukocyte subsets. *Clin. Immunol. Immunopathol.* **76**, 195–202.
29. Perretti, M., Christian, H., Wheller, S.K., Aiello, I., Mugridge, K.G., Morris, J.F., Flower, R.J., and Goulding, N.J. (2000) Annexin I is stored within gelatinase granules of human neutrophil and mobilized on the cell surface upon adhesion but not phagocytosis. *Cell. Biol. Int.* **24**, 163–174.
30. Perretti, M. and Flower, R.J. (1995) Anti-inflammatory lipocortin-derived peptides. *Agents Actions Suppl.* **46**, 131–138.
31. Perretti, M. (1997) Endogenous mediators that inhibit the leukocyte-endothelium interaction. *Trends Pharmacol. Sci.* **18**, 418–425.
32. D’Amico, M., Di Filippo, C., La, M., Solito, E., McLean, P.G., Flower, R.J., Oliani, S.M., and Perretti, M. (2000)
Lipocortin 1 reduces myocardial ischemia-reperfusion injury by affecting local leukocyte recruitment. *FASEB J.* **14**, 1867–1869.

33. Perretti, M., Ahluwalia, A., Harris, J.G., Goulding, N.J., and Flower, R.J. (1993) Lipocortin-1 fragments inhibit neutrophil accumulation and neutrophil-dependent edema in the mouse. A qualitative comparison with an anti-CD11b monoclonal antibody. *J. Immunol.* **151**, 4306–4314.

34. Gavins, F.N., Yona, S., Kamal, A.M., Flower, R.J., and Perretti, M. (2003) Leukocyte antiadhesive actions of annexin 1: ALXR- and FPR-related anti-inflammatory mechanisms. *Blood* **101**, 4140–4147.

35. Lim, L.H., Solito, E., Russo-Marie, F., Flower, R.J., and Perretti, M. (1998) Promoting detachment of neutrophils adherent to murine postcapillary venules to control inflammation: effect of lipocortin 1. *Proc. Natl. Acad. Sci. U. S. A.* **95**, 14535–14539.

36. Miele, I., Cordella-Miele, E., Facchiano, A., and Mukherjee, A.B. (1988) Novel anti-inflammatory peptides from uteroglobin and lipocortin I. *Nature* **335**, 726–730.

37. vanBinsbergen, J., Slotboom, A.I., Aarsman, A.J., and de Haas, G.H. (1989) Synthetic peptide from lipocortin I has no phospholipase A2 inhibitory activity. *FEBS Lett.* **247**, 293–297.

38. Marki, F., Pfeilschifter, J., Rink, H., and Wiesenberg, I. (1990) ‘Antiflammins’: two nonapeptide fragments of uteroglobin and lipocortin I have no phospholipase A2 inhibitory and anti-inflammatory activity. *FEBS Lett.* **264**, 171–175.

39. Zouki, C., Ouellet, S., and Filep, J.G. (2000) The anti-inflammatory peptides, antiflammins, regulate the expression of adhesion molecules on human leukocytes and prevent neutrophil adhesion to endothelial cells. *FASEB J.* **14**, 572–580.

40. Walther, A., Riehemann, K., and Gerke, V. (2000) A novel ligand of the formyl peptide receptor: annexin I regulates neutrophil extravasation by interacting with the FPR. *Mol. Cell* **5**, 831–840.

41. Ali, H., Richardson, R.M., Haribabu, B., and Snyderman, R. (1999) Chemoattractant receptor cross-desensitization. *J. Biol. Chem.* **274**, 6027–6030.

42. Durstin, M., Gao, J.L., Tiffany, H.L., McDermott, D., and Murphy, P.M. (1994) Differential expression of members of the N-formylpeptide receptor gene cluster in human phagocytes. *Biochem. Biophys. Res. Commun.* **201**, 174–179.

43. Shen, W., Proost, P., Li, B., Gong, W., Le, Y., Sargeant, R., Murphy, P.M., Van Damme, J., and Wang, J.M. (2000) Activation of the chemotactic peptide receptor FPR1 in monocytes phosphorylates the chemokine receptor CCR5 and attenuates cell responses to selected chemokines. *Biochem. Biophys. Res. Commun.* **272**, 276–283.

44. Klein, C., Paul, J.I., Sauve, K., Schmidt, M.M., Arcangeli, L., Ransom, J., Trueheart, J., Manfredi, J.P., Broach, J.R., and Murphy, A.J. (1998) Identification of surrogate agonists for the human FPR1 receptor by autocrine selection in yeast. *Nat. Biotechnol.* **16**, 1334–1337.

45. Bao, L., Gerard, N.P., Eddy, R.L., Jr., Shows, T.B., and Gerard, C. (1992) Mapping of genes for the human C5a receptor (C5AR), human FMLP receptor (FPR), and two FMLP receptor homologue orphan receptors (FPRH1, FPRH2) to chromosome 19. *Genomics* **13**, 437–440.

46. Gavins, F.N., Kamal, A.M., D’Amico, M., Oliani, S.M., and Perretti, M. (2005) Formyl-peptide receptor is not involved in the protection afforded by annexin I in murine acute myocardial infarct. *FASEB J.* **19**, 100–102.

47. Gao, J.L., Chen, H., Filie, J.D., Kozak, C.A., and Murphy, P.M. (1998) Differential expansion of the N-formylpeptide receptor gene cluster in human phagocytes. *Genomics* **51**, 270–276.

48. Wang, Z.G. and Ye, R.D. (2002) Characterization of a two new members of the formyl peptide receptor gene family from 129S6 mice. *Gene* **299**, 57–63.

49. Takano, T., Fiore, S., Maddox, J.F., Brady, H.R., Petasis, N.A., and Serhan, C.N. (1997) Aspirin-triggered 15-epi-lipoxin A4 (LXA4) and LXA4 stable analogues are potent inhibitors of acute inflammation: evidence for anti-inflammatory receptors. *J. Exp. Med.* **185**, 1693–1704.

50. Le, Y., Gong, W., Li, B., Dunlop, N.M., Shen, W., Su, S.B., Ye, R.D., and Wang, J.M. (1999) Utilization of two seven-transmembrane, G protein-coupled receptors, formyl peptide receptor-like 1 and formyl peptide receptor, by the synthetic hexapeptide WKYMVm for human phagocyte activation. *FASEB J.* **13**, 100–102.

51. Chiang, N., Takano, T., Arita, M., Watanabe, S., and Serhan, C.N. (2003) A novel rat lipoxin A4 receptor that is conserved in structure and function. *Br. J. Pharmacol.* **139**, 89–98.

52. La, M., D’Amico, M., Bandiera, S., Di Filippo, C., Oliani, S.M., Gavins, F.N., Flower, R.J., and Perretti, M. (2001) Annexin 1 peptides protect against experimental myocardial ischemia- reperfusion: analysis of their mechanism of action. *FASEB J.* **15**, 2247–2256.

53. Dreier, R., Schmid, K.W., Gerke, V., and Riehemann, K. (1998) Differential expression of annexins I, II and IV in human tissues: an immunohistochemical study. *Histochem. Cell Biol.* **110**, 137–148.

54. Oliani, S.M., Paul-Clark, M.J., Christian, H.C., Flower, R.J., and Perretti, M. (2001) Neutrophil interaction with inflamed postcapillary venule endothelium alters annexin 1 expression. *Am. J. Pathol.* **158**, 603–615.

55. Vergnolle, N., Comera, C., and Bueno, L. (1995) Annexin 1 is overexpressed and specifically secreted during experimentally induced colitis in rats. *Eur. J. Biochem.* **232**, 603–610.

56. Smith, S.F., Tetley, T.D., Guz, A., and Flower, R.J. (1990) Detection of lipocortin 1 in human lung lavage fluid: lipocortin degradation as a possible proteolytic mechanism in the control of inflammatory mediators and inflammation. *Environ. Health Perspect.* **85**, 135–144.

57. Beuckelmann, D.J., Nabauer, M., Kruger, C., and Erdmann, E. (1995) Altered diastolic[Ca2+]i handling in human
ventricular myocytes from patients with terminal heart failure. Am. Heart J. 129, 684–689.

58. Matteo, R.G. and Moravec, C.S. (2000) Immunolocalization of annexins IV, V and VI in the failing and non-failing human heart. Cardiovasc. Res. 45, 961–970.

59. Ritchie, R.H., Sun, X., Bilszta, J.L., Gulliyan, L.M., and Dusting, G.J. (2003) Cardioprotective actions of an N-terminal fragment of annexin-1 in rat myocardium in vitro. Eur. J. Pharmacol. 461, 171–179.

60. Willmott, N.J., Choudhury, Q., and Flower, R.J. (1997) Effects of dexamethasone and phorbol ester on P2 receptor-coupled Ca2+ signalling and lipocortin 1 presentation in U937 cells. Br. J. Pharmacol. 122, 1055–1060.

61. Reilly, A.M., Sun, X., Williams, D.A., and Dusting, G.J. (1999) Dexamethasone inhibits endotoxin-induced changes in calcium and contractility in rat isolated papillary muscle. Cell Calcium 26, 1–8.

62. Lipton, J.M. and Catania, A. (1997) Anti-inflammatory actions of the neuroimmunomodulator alpha-MSH. Immunol. Today 18, 140–145.

63. Heinig, J.A., Keeley, F.W., Robson, P., Sower, S.A., and Youson, J.H. (1995) The appearance of proopiomelanocortin early in vertebrate evolution: cloning and sequencing of POMC from a Lamprey pituitary cDNA library. Gen. Comp. Endocrinol. 99, 137–144.

64. Wikberg, J.E., Muceniece, R., Mandrika, I., Prusis, P., Lindblom, J., Post, C., and Skottner, A. (2000) New aspects on the melanocortins and their receptors. Pharmacol. Rev. 42, 393–420.

65. Castro, M.G. and Morrison, E. (1997) Post-translational processing of proopiomelanocortin in the pituitary and in the brain. Crit. Rev. Neurobiol. 11(1), 35–57.

66. von Eggelkraut-Gottanka, R. and Beck-Sickinger, A.G. (2004) Biosynthesis of peptide hormones derived from precursor sequences. Curr. Med. Chem. 11, 2651–2665.

67. Benjannet, S., Rondeau, N., Day, R., Chretien, M., and Seidah, N.G. (1991) PC1 and PC2 are proprotein convertases capable of cleaving proopiomelanocortin at distinct pairs of basic residues. Proc. Natl. Acad. Sci. U. S. A. 88, 3564–3568.

68. Catania, A., Gatti, S., Colombo, G., and Lipton, J.M. (2004) Targeting melanocortin receptors as a novel strategy to control inflammation. Pharmacol. Rev. 56, 1–29.

69. Star, R.A., Rajora, N., Huang, J., Stock, R.C., Catania, A., and Lipton, J.M. (1995) Evidence of autocrine modulation of macrophage nitric oxide synthase by α-melanocyte-stimulating hormone. Proc. Natl. Acad. Sci. U. S. A. 92, 8016–8020.

70. Taherzadeh, S., Sharma, S., Chhajlani, V., Gantz, I., Rajora, N., Demitri, M.T., Kelly, I., Zhao, H., Ichiyama, T., Catania, A., and Lipton, J.M. (1999) α-MSH and its receptors in regulation of tumour necrosis factor-a production by human monocyte/macrophages. Am. J. Physiol. 276, R1289–R1294.

71. Bhardwaj, R., Becher, E., Mahnke, K., Hartmeyer, M., Schwarz, T., Scholzen, T., and Luger, T.A. (1997) Evidence for the differential expression of the functional alpha-melanocyte-stimulating hormone receptor MC-1 on human monocytes. J. Immunol. 158, 3378–3384.

72. Catania, A., Rajora, N., Capsooni, F., Minonzio, F., Star, R.A., and Lipton, J.M. (1996) The neuropeptide α-MSH has specific receptors on neutrophils and reduces chemotaxis in vitro. Peptides 17(4), 675–679.

73. Hartmeyer, M., Scholzen, T., Becher, E., Bhardwaj, R.S., Schwarz, T., and Luger, T.A. (1997) Human dermal microvascular endothelial cells express the melanocortin receptor type 1 and produce increased levels of IL-8 upon stimulation with alpha-melanocyte-stimulating hormone. J. Immunol. 159, 1930–1937.

74. Bohm, M., Schulte, U., Kalden, H., and Luger, T.A. (1999) Alpha-melanocyte-stimulating hormone modulates activation of NF-kappa B and AP-1 and secretion of interleukin-8 in human dermal fibroblasts. Ann. N Y Acad. Sci. 885, 277–286.

75. Adachi, S., Nakano, T., Vliagoftis, H., and Metcalfe, D.D. (1999) Receptor-mediated modulation of murine mast cell function by alpha-melanocyte-stimulating hormone. J. Immunol. 163, 3363–3368.

76. Neumann Andersen, G., Nagaeva, O., Mandrika, I., Petrovska, R., Muceniece, R., Mincheva-Nilsson, L., and Wikberg, J.E. (2001) MC(1) receptors are constitutively expressed on leucocyte subpopulations with antigen presenting and cytotoxic functions. Clin. Exp. Immunol. 126, 441–446.

77. Xia, Y., Wikberg, J.E., and Chhajlani, V. (1995) Expression of melanocortin 1 receptor in periaqueductal gray matter. Neuroreport 6, 2193–2196.

78. Penhoat, A., Jaillard, C., and Saez, J.M. (1989) Corticotropin positively regulates its own receptors and cAMP response in cultured bovine adrenal cells. Proc. Natl. Acad. Sci. U. S. A. 86, 4978–4981.

79. Boston, B.A. and Cone, R.D. (1996) Characterization of melanocortin receptor subtype expression in murine adipose tissues and in the 3T3-L1 cell line. Endocrinology 137(5), 2043–2050.

80. Chhajlani, V. (1996) Distribution of cDNA for melanocortin receptor subtypes in human tissues. Biochem. Mol. Biol. Int. 38, 73–80.

81. Gantz, I., Konda, Y., Tashiro, T., Shimoto, Y., Miwa, H., Munzert, G., Watson, S.J., DelValle, J., and Yamada, T. (1993) Molecular cloning of a novel melanocortin receptor. J. Biol. Chem. 268(11), 8246–8250.

82. Butler, A.A., Kesterson, R.A., Khong, K., Cullen, M.J., Pellemounter, M.A., Dekoning, J., Baetscher, M., and Cone, R.D. (2000) A unique metabolic syndrome causes obesity in the melanocortin-3 receptor-deficient mouse. Endocrinology 141, 3518–3521.

83. Getting, S.J., Gibbs, L., Clark, A.J.L., Flower, R.J., and Perretti, M. (1999) POMC gene derived peptides activate melanocortin type 3 receptor on murine macrophages, suppress cytokine release and inhibit neutrophil migration in...
acute experimental inflammation. J. Immunol. 162, 7446–7453.

84. Getting, S.J., Allcock, G.H., Flower, R., and Perretti, M. (2001) Natural and synthetic agonists of the melanocortin receptor type 3 possess anti-inflammatory properties. J. Leukoc. Biol. 69, 98–104.

85. Getting, S.J., Christian, H.C., Lam, C.W., Gavins, F.N., Flower, R.J., Schioth, H.B., and Perretti, M. (2003) Redundancy of a functional melanocortin 1 receptor in the anti-inflammatory actions of melanocortin peptides: studies in the recessive yellow (e/e) mouse suggest an important role for melanocortin 3 receptor. J. Immunol. 170, 3323–3330.

86. Getting, S.J., Christian, H.C., Flower, R.J., and Perretti, M. (2002) Activation of melanocortin type 3 receptor as a molecular mechanism for adrenocorticotropic hormone efficacy in gouty arthritis. Arthritis Rheum. 46, 2765–2775.

87. Lam, C.W., Getting, S.J., and Perretti, M. (2005) In vitro and in vivo induction of heme oxygenase 1 in mouse macrophages following melanocortin receptor activation. J. Immunol. 174, 2297–2304.

88. Guarini, S., Schioth, H.B., Mioni, C., Cainazzo, M., Ferarra, G., Giuliani, D., Wikberg, J.E., and Bertolini, A. (2000) D-Amino acid scan of gamma-melanocyte-stimulating hormone: importance of Trp(8) on human MC3 receptor selectivity. J. Med. Chem. 43, 4998–5002.

89. Mioni, C., Giuliani, D., Cainazzo, M.M., Leone, S., Iannone, C., Bazzani, C., Grieco, P., Novellino, E., Tomasi, A., Bertolini, A., and Guarini, S. (2003) Further evidence that melanocortins prevent myocardial reperfusion injury by activating melanocortin MC3 receptors. Eur. J. Pharmacol. 477, 227–234.

90. Robbins, L., Nadeau, J.H., Johnson, K.R., Kelly, M.A., Roselli-Rehfuss, L., Taft, G., Michael, L.H., and Entman, M.L. (2003) Development of murine ischemic cardiomyopathy is associated with a transient inflammatory reaction and depends on reactive oxygen species. Proc. Natl. Acad. Sci. U. S. A. 100, 2700–2705.

91. Lawrence, T., Willoughby, D.A., and Gilroy, D.W. (2002) Anti-inflammatory lipid mediators and insights into the resolution of inflammation. Nat. Rev. Immunol. 2, 787–795.

92. Granger, D.N. (1999) Ischemia-reperfusion: mechanisms of microvascular dysfunction and the influence of risk factors for cardiovascular disease. Microcirculation 6, 167–178.

93. Dewald, O., Frangogiannis, N.G., Zerlein, M., Duer, G.D., Klemm, C., Kneuefmann, P., Taffet, G., Michael, L.H., Crapo, J.D., Welz, A., and Entman, M.L. (2003) Development of murine ischemic cardiomyopathy is associated with a transient inflammatory reaction and depends on reactive oxygen species. Proc. Natl. Acad. Sci. U. S. A. 100, 2700–2705.

94. Taylor, A. and Namba, K. (2001) In vitro induction of CD25+ CD4+ regulatory T cells by the neuropeptide alpha-melanocyte-stimulating hormone (alpha-MSH). Immunol. Cell Biol. 79, 358–367.

95. Getting, S. (2002) Melanocortin peptides and their receptors: new targets for anti-inflammatory therapy. Trends Pharmacol. Sci. 23, 447.

96. Buggy, J.J. (1998) Binding of alpha-melanocortin-stimulating hormone to its G-protein-coupled receptor on B-lymphocytes activates the Jak/STAT pathway. Biochem J. 331(Pt 1), 211–216.

97. Taylor, A. and Namba, K. (2001) In vitro induction of CD25+ CD4+ regulatory T cells by the neuropeptide alpha-melanocyte stimulating hormone (alpha-MSH). Immunol. Cell Biol. 79, 358–367.

98. Szardenings, M., Muceniece, R., Mutule, I., Mutulis, F., and Wikberg, J.E. (2000) New highly specific agonistic melanocortin 1 receptor. Peptides 21, 239–243.

99. Kask, A., Rago, L., Mutulis, F., Pahkla, R., Wikberg, J.E., and Schioth, H.B. (1998) Selective antagonist for the melanocortin 4 receptor (HS014) increases food intake in free-feeding rats. Biochem. Biophys. Res. Commun. 245, 90–93.

100. Mioni, C., Giuliani, D., Cainazzo, M.M., Leone, S., Iannone, C., Bazzani, C., Grieco, P., Novellino, E., Tomasi, A., Bertolini, A., and Guarini, S. (2003) Further evidence that melanocortins prevent myocardial reperfusion injury by activating melanocortin MC3 receptors. Eur. J. Pharmacol. 477, 227–234.

101. Robbins, L.S., Nadeau, J.H., Johnson, K.R., Kelly, M.A., Roselli-Rehfuss, L., Taft, G., Michael, L.H., Crapo, J.D., Welz, A., and Entman, M.L. (2003) Development of murine ischemic cardiomyopathy is associated with a transient inflammatory reaction and depends on reactive oxygen species. Proc. Natl. Acad. Sci. U. S. A. 100, 2700–2705.

102. Gavins et al.: Annexin and Melanocortin ischaemic-reperfusion damage in the heart. TheScientificWorldJournal 6, 1008–1023. DOI 10.1100/tsw.2006.196.