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COMMENTARY

Attractin' more attention – new pieces in the obesity puzzle?

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Genetic, biochemical and pharmacological studies in humans and rodents have established that signalling through the G-protein-coupled melanocortin-4 receptor (MC4R) by pro-opiomelanocortin (POMC)-derived ligands plays a critical role in the central suppression of appetite. As a consequence, malfunction of this signalling system leads to the development of obesity. It has been shown previously that melanocortin signalling can be modulated by the type 1 transmembrane protein attractin, apparently acting as a co-receptor for the inhibitory ligand agouti. Work reported in this issue of *Biochemical Journal* (Haqq et al.) demonstrates that the cytosolic tail of an attractin-like protein (ALP) binds directly and specifically to the C-terminal region of MC4R, raising the possibility that proteins of the attractin family influence melanocortin receptor function through multiple mechanisms.

Key words: agouti, appetite, attractin, G-protein-coupled receptor (GPCR), melanocortin, obesity.

Obesity is now a pandemic of global proportions, affecting particularly the 'developed' world and, increasingly, the 'near-developed' world. According to the World Health Organization (http://www.who.int), as many as 8 % of the adult world population are clinically obese, as defined by a BMI [body mass index: weight in kg/(height in m)^2] of >30 kg/m^2. In the United States, the proportion of obese adults has increased from 15 % to 25 % over the last 20 years. With this rise in the prevalence of obesity comes the associated baggage of co-morbidities, including increased incidences of Type II diabetes, cardiovascular disease, hypertension and certain cancers. It is no wonder that new findings in obesity research continue to engender great interest both among the scientific community and with the public at large. Of course, it is a truism to say that no-one ever became fat except by eating too much. But it is now clear that when it comes to controlling our appetites we do not have as much free will as we might like to believe, but rather are driven to eat by powerful biochemical forces which are at least in part genetically determined. We now know that the melanocortin pathway plays a key role in the control of food intake and body weight, and there is compelling evidence that disruption of hypothalamic melanocortin signalling results in severe obesity.

The diverse functions that have been attributed to the melanocortin peptides, α-, β- and γ-melanocyte-stimulating hormones, embrace melanogenesis, steroidogenesis, sexual function and inflammation, as well as appetite regulation and energy homoeostasis [1]. These actions are mediated by a family of five melanocortin receptors (MCRs: MC1R–MC5R), which are expressed in a tissue-specific manner. Several lines of genetic evidence indicate that signalling through the MC4R exerts an inhibitory influence on appetite, and thus on the development of obesity (reviewed in [2,3]). Both humans and mice with a deficiency of pro-opiomelanocortin (POMC), the propeptide precursor of melanocortins, are severely obese. Likewise, humans and mice carrying mutations in the prohormone convertases that are responsible for the processing of POMC are obese. Abrogation of signalling through the MC4R, either by disruption of the gene encoding the MC4R itself or by ectopic overexpression of antagonists, also causes severe obesity. The downstream targets of MC4R signalling have until recently remained obscure. However, earlier this year it was reported that signalling by the MC4R in the ventromedial nucleus of the hypothalamus induces the expression of brain-derived neurotrophic factor (BDNF), which goes on to signal to the TrkB receptor [4]. Homozygous knock-outs of *Bdnf* or *Trkb* genes are lethal, but less severely reduced expression of these molecules leads to obesity.

It is now widely accepted therefore that the central melanocortin pathway is critical in appetite regulation, and that the activity of the MC4R plays a pivotal role in the development of obesity. Strikingly, mice that are homozygous for a targeted disruption of their *Mc4r* gene exhibit a severe obesity syndrome characterized by hyperphagia, hyperinsulinaemia, hyperglycaemia and increased linear growth, with no abnormality of the reproductive or adrenal axes. However, relatively modest changes in MC4R number or signalling capacity can also be important for appetite control and development of obesity. Heterozygous *Mc4r*$^{+/-}$ mice have an intermediate phenotype when compared with their homozygous *Mc4r*$^{-/-}$ and wild-type littermates [5]. Likewise, in humans, where mutations in the MC4R account for up to 6 % of cases of severe early-onset obesity, most of the mutations are found in heterozygous form. Remarkably, the activity of mutant receptors assessed in reporter assays *in vitro* can be correlated with the appetite of individuals carrying the mutations [6]. Such studies suggest that quite subtle pathophysiological modulation of MC4R function might influence appetite and tendency to obesity.

The MC4R is a typical member of the GPCR (G-protein-coupled receptor) superfamily, and signals by interaction with G_s and activation of adenylate cyclase. GPCRs are a very large family of proteins represented by several hundred genes in the human genome. In addition to the heterotrimeric G-proteins that relay intracellular signals from GPCRs, there are several classes of proteins that interact with specific GPCRs, modulating their function. The phenomenon of homologous agonist-induced desensitization is well known and widespread among GPCRs. It is triggered by agonist-induced phosphorylation, catalysed by GPCR kinases (GRKs), on serine/threonine residues within the C-terminal segment and third intracellular loop of receptors. Phosphorylation promotes binding of β-arrestins, which modulate function of many different GPCRs [7]. Arrestins were originally seen as a negative influence, attenuating signalling both by uncoupling receptors from G-proteins and by targeting receptors to clathrin-coated pits for internalization and, potentially,

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lysosomal degradation. Recent work has shown that MC4R conforms to this pattern and undergoes agonist-mediated desensitization and internalization through GRK-, β-arrestin- and dynamin-dependent processes [8].

It is now recognized that β-arrestins can also function positively as scaffold proteins that couple receptors to MAPK (mitogen-activated protein kinase)-signalling pathways involving ERKs (extracellular-signal-regulated kinases) and/or JNKs (c-Jun N-terminal kinases), and thus direct new signals from the very receptors they desensitize in terms of G-protein-mediated signalling [7]. Moreover, β-arrestins are by no means the only scaffolds that have been demonstrated to interact with GPCRs. Numerous different cytosolic proteins have been shown to bind to GPCRs, including PDZ-domain proteins, protein kinases and cytoskeletal proteins [9,10]. Binding is generally sequence-specific, commonly involving the C-terminal tail of receptors, and therefore particular scaffolds associate with particular receptors. Such interactions have the potential to modulate function in various ways, including allosteric regulation of signalling, clustering with potential effectors or targeting to specific subcellular compartments or plasma membrane domains.

There is little published information on proteins that interact with melanocortin receptors. One such protein, attractin, was identified as the product of the murine *Mahogany* gene [11]. *Mahogany* mutations that impair attractin function are associated with altered pigmentation and with obesity, indicating that attractin can modulate function of both MC1R and MC4R. Unlike most previously studied GPCR-interacting proteins, attractin is not cytosolic, but a type 1 transmembrane protein of 1428 amino acids with a large extracellular domain and a relatively short cytosolic tail of 128 amino acids. It appears to function as a co-receptor for the endogenous MCR-inhibitory protein agouti [12] (but not the agouti-related protein AGRP, which had been previously implicated in appetite regulation [13]). However, the mechanism of interaction of attractin with MCRs is not altogether clear. Further complexity is introduced, as if it were needed, by the existence of isoforms of attractin with different C-termini, which are secreted, rather than transmembrane, proteins. One other example of a transmembrane protein which modulates GPCR function is the D_1 dopamine receptor-interacting protein calcyon [14]. This is a protein of only 24 kDa, which apparently regulates the affinity state of receptors as well as cross-talk between G_s-coupled D_1 receptors and heterologous G_q-coupled receptors. Again, the mechanism of interaction between this regulatory protein and the D_1 receptor has not been delineated at a molecular level.

Into the fray then come a group of MC4R investigators from the Vollum Institute in Portland, OR, U.S.A., and their collaborators from Harvard Medical School and the Rockefeller University. In this issue of the *Biochemical Journal*, Haqq et al. provide tantalizing evidence for a novel binding partner for the MC4R identified in a yeast two-hybrid screen of a mouse brain cDNA library [15]. Out of this screen popped an attractin-like protein (ALP), apparently the murine orthologue of a human protein for which the sequence was already known (KIAA0534). ALP is a transmembrane protein of 1371 amino acids (KIAA0534 has just 1175 amino acids) and its cytosolic tail is 63 % identical in sequence with the corresponding region of mouse attractin-1. Nothing is divulged by Haqq et al. about the structure of the extracellular domain of ALP and its relationship to attractin. However, the ALP–MC4R interaction is mapped to specific regions of the cytosolic C-terminal tail of ALP and the C-terminal tail of MC4R (spanning Thr^{312}, one of the phosphorylation sites implicated in β-arrestin binding and agonist-induced desensitization [8]). There is also evidence of specificity, in that the MC4R tail binds the C-terminus of ALP but not attractin, whereas the C-terminus of ALP binds MC4R but not β_2-adrenergic receptor. Many interesting details remain to be addressed, not least whether ALP interacts with other MCRs and whether the interaction is affected by receptor phosphorylation. No data are presented on the functional consequences of the ALP–MC4R interaction, but circumstantial evidence of physiological significance is provided by the co-localization of ALP and MC4R in a number of brain regions known to be important to the regulation of energy homoeostasis, although expression of ALP is by no means confined to such sites. Precisely what the physiological function of ALP might be is unclear at this stage. The authors speculate that it might act as a co-receptor for AGRP, or otherwise facilitate inhibition of MC4R function by AGRP, in a manner analogous to the role of attractin in mediating effects of agouti. However, other roles of ALP in clustering and synaptic localization of MC4R are not ruled out. The demonstration that attractin is but one of a family of related proteins capable of interacting with melanocortin receptors is certain to act as a 'spur' to further studies designed to elucidate the physiological consequences of such interactions, and the biochemical mechanisms underlying them.

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