New therapeutic developments in the regulation of food intake by neuropeptides
Experience from studies with CART and GLP-2

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BACKGROUND

Over the last decade neuropeptides have gained increasing interest as potential drug targets in both central and peripheral neuronal pathways as well as in neuroendocrine systems. In contrast to the almost ubiquitous nature of classic neurotransmitters, neuropeptides are often more distinctly expressed in the CNS. Thus, a large proportion of neuropeptidergic pathways exhibit restricted distribution with selective target areas. The pharmacological responses of target areas are further specified by the existence of multiple receptor subtypes for each of the neuropeptides. It has been clear for many years that most neurons co-express classic neurotransmitters with one or several neuropeptides. However, neuroendocrine neurons tend to express only neuropeptides but with such diversity that the functional significance is difficult to decipher. The presence of multiple neurotransmitters in individual peptidergic neurons emphasizes the need to turn functional focus from singularity to diversity. Neuropeptides exert modulatory functions with spatial and temporal effects distinctly different from classic small molecule transmitters. Long-term impacts of neuropeptides on target cells are also diverse ranging from hours of altered firing patterns to trophic functions. Most classic neurotransmitters mediate their actions via wiring transmission i.e. in serial connectivity patterns, but neuropeptides may also function as volume transmitters mediating their actions via parallel couplings with high divergence having impact on numerous targets. Thus, volume-transmitting neuropeptides typically exert tonic effects on targets, whereas wiring transmitting neurotransmitters exert phasic effects on their targets. A classic example of volume transmission is the tonic effects of neuropeptide Y on sympathetic nervous activity but neuropeptide volume transmission has also been demonstrated in the central nervous system.

Neuropeptidergic systems are particularly present in limbic structures governing adaptive behaviours and rheostatic mechanisms. Mediation of both higher order limbic functions (affective biasing of sensory input, motivational states, integrated motor behaviour) and primary affects (hunger, thirst, thermoregulation, thermogenesis) relies to a large extent on neuropeptidergic transmission. Neuropeptides are also abundantly expressed in neurons constituting both the submucose and myenteric plexuses as well as in enteroendocrine cells of the gastrointestinal tract. Thereby, neuropeptides influence a broad spectrum of gastrointestinal functions including those with an impact on absorption of nutrients. It is hardly surprising that neuropeptides have attracted intense interest from researchers studying the neuronal pathways involved in regulation of food intake and energy homeostasis. Given the immense need for better pharmaceutical strategies towards treatment of obesity, the knowledge obtained from basic science has inspired a wealth of pharmaceutical companies to set up research programmes identifying therapeutic principles targeting neuropeptidergic pathways. A long list of neuropeptides with either anabolic or catabolic effects exists and more candidates are continuously added.

Obviously, neuropeptides need not necessarily to be part of an endogenous energy rheostat to constitute attractive pharmaceutical targets. However, the likelihood of therapeutic success is probably higher if neuropeptide drug targets are known satiety factors, stimulators of feeding or regulators of metabolic rate. Most of the aforementioned neuropeptides have been extensively studied and qualify fully for a regulatory role in the physiology of energy homeostasis. Some of these neuropeptides are regulated by circulating hormones reflecting energy stores in adipocytes (leptin and insulin), such that catabolic factors are expressed in elevated quantities in obese subjects and at lower levels in lean subjects. However, also short-term fluctuation of leptin,
insulin and glucose levels influence neuropeptide gene expression in hypothalamic nuclei involved in regulation of feeding. Although very few studies have shown that neuropeptides with an impact on body energy homeostasis are released at specific target sites upon relevant physiologic stimuli, their functional importance has been substantiated by other direct means as well as by circumstantial evidence.

Most pharmacological experiments includes that a relevant behaviour or clinical end point is eliminated by prior administration of a receptor specific antagonist. With the advent of more specific ligands this strategy is viable for most neuropeptide systems but clear cut evidence of participating receptors may be hampered by the involvement of more than one receptor in mediating a given behaviour as is seen for neuropeptide Y. At other instances, the absence of an identified putative receptor as seen for CART renders pharmacological characterization of analogues impossible. Genetically modified animals with specific knock out of either neuropeptide encoding genes or their receptor encoding genes have helped to clarify the involvement of some neuropeptides in feeding behaviour. Thus, mice with a genetic knock out of MCH are skinny whereas mice over-expressing MCH develop an obese glucose intolerant phenotype [1, 2]. The tight inverse correlation between circulating leptin levels and MCH synthesis in neurones of the lateral hypothalamus further strengthens that MCH is an anabolic neuropeptide with stimulatory influence on feeding and reduced energy expenditure. The MCH neurones of the LHA co-localise the anorectic neurotransmitter CART but it the functional consequence of this co-existence is unknown [3]. Leptin levels correlate negatively to hypothalamic MCH and positively to hypothalamic CART levels suggesting that the ratio of co-released MCH/CART depends on available energy stores such that MCH prevails at states of energy deficit. Expression of NPY in neurones of the arcuate nucleus is also enhanced when circulating levels of leptin are lowered, and numerous studies including experiments on non-human primates have shown that NPY is a powerful mediator of starvation induced feeding [4]. However, the apparent lack of a phenotype in NPY knock out mice has made some scientist erroneously to conclude that NPY is irrelevant as a long-term regulator of feeding. The anticipated development of a skinny phenotype in NPY knock out mice becomes evident only when NPY knock out mice are crossed with leptin deficient ob/ob mice emphasising the redundancy of neuropeptide systems regulating feeding behaviour [5]. The NPY system apparently mediates its actions via multiple receptors as evidenced by small but significant changes of energy homeostasis in some groups of Y1, Y2 and Y5 knock out mice.

When assessing the possible role of a neuropeptide as a neurotransmitter with an impact on body weight homeostasis it is pertinent to determine biological site of action, which imply a neuroanatomical characterisation of the system in question. The emerging concept of energy regulating pathways places a number of hypothalamic nuclei as central players (fig. 1). Long-term information about body energy stores (adipocyte lipid content) is primarily sensed by interaction of leptin and insulin with neurones of the arcuate nucleus. However, also neurones of the ventromedial and dorsomedial hypothalamic nuclei are sensitive to leptin. Short-term deviations of energy availability are mediated by glucose

Figure 1 : Neuroanatomy of the ponderostat (areas involved in feeding behaviour and energy homeostasis).

Figure 1 : L'anatomie neurologique des pondérotats (zones impliquées dans le comportement alimentaire et l'homéostase énergétique.)
to glucose responding neurones of the arcuate and ventromedial nuclei. Online registration of gastro-intestinal function including filling, peristalsis and nutrient absorption is conveyed to the lower brainstem by vagal afferents as well as by circulating GI tract hormones. Most of the transmitters of this gut-brain axis are neuropeptides and interestingly, the transmitters synthesised in ascending pathways connecting the nucleus of the solitary tract to hypothalamic target areas are often identical to those released by gastro-intestinal enteroendocrine cells raising interesting comparative aspects of the development of neural control of hunger and satiety.

The impermeability of both the blood brain barrier and the gastrointestinal lining to most peptides obviously challenge medicinal chemistry in mimicking peptidergic ligands. However, several small molecule non-peptidergic analogues have been synthesised and it should be possible to invent both specific antagonists as well as agonist for all known receptor proteins involved in energy homeostasis. Before engaging in such endeavours, it may prove beneficial to verify that the satiating effect of the treatment is specific and that long-term treatment with a given agonist/antagonist is not subject to tachyphylaxis. Unequivocal specificity of a given behaviour induced by administration of exogenous neuropeptides may be extremely difficult to demonstrate because some neuropeptides are expressed in a multitude of central pathways. Therefore, it is entirely possible that a specific neurotransmitter may induce non-specific as well as specific behaviours resulting in decreased feeding. Such an example is given by CRH, which in addition to decreased food intake also enhances anxiety levels in several behavioural models. However, by means of microinjections into anatomically defined areas it has been demonstrated that the anorectic response to CRH is primarily elicited by CRH type 2 receptors in the PVN whereas CRH type 1 receptors in the dorsal periaqueductal gray mediate the anxiogenic effects of this neuropeptide [6, 7].

OWN STUDIES

Others and we have gained evidence that cocaine amphetamine regulated transcript is present in several of hypothalamic areas involved in feeding behaviour. The expression of CART in the arcuate nucleus is regulated by leptin as evidenced by the complete lack of CART in animals with deficient leptin signalling [8]. We have been unable to validate earlier studies showing cocaine and amphetamine regulated expression of CART mRNA in the accumbens and striatum. Acute intracerebroventricular (icv) administration of recombinant CART(42-89) dose-dependently diminishes nocturnal feeding and starvation induced feeding in rats (effective doses: 1-10 µg). However, doses above 2.5 µg also cause motor disturbances with profound resemblance of ataxia rendering unequivocal determination of behavioural specificity difficult. However, it is possible to differentiate between anorectic and motor effects, because microinjections of picomolar doses of CART(42-89) into the PVN have shown that anorexia is elicited without concomitant ataxia. The long-term therapeutic effects of CART(42-89) have been assessed in both lean and obese Zucker rats. Animals subjected to either of two doses of CART(42-89) for ten days ate less and lost body weight in the initial 4-5 days of the treatment period but tachyphylaxis developed and during the last 5 days of the infusion period food intake was similar in vehicle and CART(42-89) treated rats [9]. Recent data obtained from CART knock out mice also suggest they have normal energy homeostasis as evidenced by their normal lean phenotype. The lack of an identified CART receptor makes pharmacotherapeutic advances in the area of CART neurotransmission very difficult because the obvious developmental tool for medicinal chemistry is missing.

In contrast to CART, the receptor for glucagon-like peptide-1(7-36) (GLP-1) has been known for more than a decade. Most of the pharmacological studies on GLP-1 have focused upon its role as an incretin, but several acute infusion studies on human volunteers have

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**Table I**

Neuropeptides serving as transmitters in central pathways involved energy homeostasis classified as anabolic or catabolic.

<table>
<thead>
<tr>
<th>ANABOLIC</th>
<th>CATABOLIC</th>
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<tbody>
<tr>
<td>Neuropeptide Y</td>
<td>α-MSH</td>
</tr>
<tr>
<td>Agouti related protein (AGRP)</td>
<td>Corticotrophin-releasing hormone (CRH)</td>
</tr>
<tr>
<td>Melanin-concentrating hormone (MCH)</td>
<td>Urocortin</td>
</tr>
<tr>
<td>Orexin/hypocretin</td>
<td>Cholecystokinin (CCK)</td>
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<tr>
<td>Galanin</td>
<td>Glucagon related peptide-1 (GLP-1)</td>
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<tr>
<td>Ghrelin</td>
<td>Glucagon related peptide-2 (GLP-2)</td>
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<tr>
<td>Growth hormone</td>
<td>Neurotensin</td>
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<tr>
<td>β-Endorphin</td>
<td>Oxytocin</td>
</tr>
<tr>
<td>Anandamide (endogenous cannabinoids)</td>
<td>Bombesin like peptides (GRP)</td>
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<tr>
<td></td>
<td>Somatostatin</td>
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shown that GLP-1 inhibits gastric emptying with resultant subjective feeling of satiety and decreased hunger. Assessment of caloric intake has shown that GLP-1 infused humans eat less energy in single meals, but long-term studies are awaited to fully support that peripherally acting GLP-1 analogues may be used as weight reducing agents [10]. However, we have used a stable peptidergic GLP-1 derivative, NN2211, in a subchronic study on rats. Rats treated for 10 days with the GLP-1 derivative ate approximately 65 % of the food ingested by vehicle treated rats with a resulting decreased body weight (85 % of pretreatment levels). Animals were followed after cessation of the treatment and lepint measurements confirmed that adipose tissue was lost as a result of the treatment. These data clearly suggest that peripherally acting GLP-1 agonists may prove valuable as weight reducing agents in particular in overweight type 2 diabetic patients because of its beneficial effects on glycaemic control [11].

Others and we have also given evidence that central administration of GLP-1 lowers food intake. Site directed injections and lesion studies gives evidence that GLP-1 elicits its specific anorectic actions via neureones in the PVN and the arcuate nucleus [12]. The lack of non-peptidergic or stable soluble peptide analogues have made chronic icv infusion studies very difficult but a few studies employing repetitive injections have shown that GLP-1 infers lasting anorexia upon central injection and that repetitive administration of the GLP-1 antagonist exendin-9-39 actually enhance food intake with resultant increase of body weight. However, a series of studies on rats have given evidence that besides specific actions on the rheostat involved in body energy homeostasis, GLP-1 also impacts circuits involved in aversive behaviour [13]. Rats like other rodents do not vomit upon ingestion of potentially harmful agents and have possibly evolved a very sensitive taste aversive behaviour including ingestion of neutralising soil (pica). GLP-1 acts as a neural transmitter in this pathway, which involves GLP-1 receptors in the parabrachial and central amygdala nuclei. At present it is unclear whether humans as well as non-human primates possess a similar central GLP-1 pathway mediating gastrointestinal discomfort, but our own preliminary experiments with non-human primates have shown that administration of high doses of GLP-1 into the third cerebral ventricle induce anorexia but no vomiting. In the lower brainstem neureones expressing the hypothalamic neuropeptide GLP-2 containing nerve fibres to the dorsomedial hypothalamus support that this pathway may involve in aversive behaviour. GLP-2 dose-dependently decrease food intake from ingestion of a meal. However, more experiments are needed to fully justify this suggestion.

Recent anatomical and physiological studies of the co-expressed neuropeptide GLP-2 have revealed the presence of highly unique neuronal circuit. Central administration of GLP-2 dose-dependently decrease food intake in rats [14]. Receptors for GLP-2 are expressed exclusively in a small population of hypothalamic neureones residing in the compact portion of the dorsomedial nucleus. Ascending GLP-1/GLP-2 containing nerve fibres from the nucleus of the solitary tract heavily innervates the dendritic aborizations of these neureones. Studies of c-fos expression elicited by central GLP-2 administration show that these neureones are the only activated by the neuropeptide. The dorsomedial nucleus is centrally involved in regulation of energy homeostasis and lessons learned from lesion experiments suggest that this nucleus participates in defining the lower threshold of the gain of the ponderostat (whatever anatomical substrate that may have). The presence of a highly targeted organisation of GLP-2 containing nerve fibres to the dorsomedial hypothalarnus support that this pathway may have specific impact on the gain of the ponderostat probably by inhibiting the output of the DMH.

In conclusion, several peptidergic systems both in the periphery and in the central nervous system constitute pharmacological targets for weight reducing therapy. Already, a number of non-peptidergic compounds having impact on these systems are in pre-clinical development. In the near future, the obese patients will tell us if the drugs keep their promises.

REFERENCES

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