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Ups and downs for neuropeptides in body weight homeostasis: pharmacological potential of cocaine amphetamine regulated transcript and pre-proglucagon-derived peptides

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Abstract

Although most humans experience an underlying upwards drift of the body-weight set-point, body weight appears tightly regulated throughout life. The present review describes the structural basis of the adipostat and hypothesise, which components may constitute available targets for pharmacotherapy of excess body weight. Hypothalamic neurones constitute the major components of the body weight homeostasis maintaining device. Together with neurones of the nucleus of the solitary tract, neurones of the hypothalamic arcuate nucleus constitute the sensory components of the adipostat. The arcuate nucleus neurones respond to circulating levels of leptin and insulin, both of which reflect the levels of energy stored as triacylglycerol in adipocytes. The arcuate nucleus projects heavily to the hypothalamic paraventricular nucleus. Neurones of the hypothalamic paraventricular nucleus are hypothesised to constitute, at least partly, the adipostat motor pattern generator, which upon stimulation activates either net anabolic or catabolic physiological responses. The overall sensitivity of the adipostat is influenced by gain setting neurones hypothesised to be located in the dorsomedial hypothalamic nucleus and lateral hypothalamic area. Cocaine amphetamine regulated transcript (CART) peptides and pre-proglucagon derived peptides, glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2) are catabolic neurotransmitters synthesised in neurones of the arcuate nucleus and the nucleus of the solitary tract, respectively. The present review summarises the available evidence that both families of peptides constitute endogenous transmitters mediating satiety and touch upon potential pharmacological exploitation of this knowledge.

Keywords: Adipostat; Leptin; Hypothalamus; Obesity; Appetite; Satiety

1. Introduction

Chronic diseases call for good medical care and rational pharmacotherapy. The avenues to development of rational treatment paradigms of chronic diseases, as exemplified by long recognised diseases as arterial hypertension and type 2 diabetes, are long and winding as we are still awaiting drugs with lesser side effects and better efficacy. Historically, only few pharmacological therapies to alleviate obesity and its co-morbidities have been introduced, and nearly all therapies have been terminated prematurely due to disastrous side effects (addiction with amphetamine analogues, pulmonary hypertension in Aminorex treated individuals, Valvular heart disease in fenfluramine treated patients, and neuropathy and cataracts in dinitrophenol treatment). Over the last decade, efforts to develop specific and safe anti-obesity drugs have therefore increased tremendously. Today, a large number of basic scientists in academia as well as industry employed scientist tinker with practically all of nature's handles having a potential impact on body weight homeostasis. It is often claimed that the cloning and subsequent synthesis of the adipocyte derived hormone leptin spurred a phenomenal research interest in the field of obesity. However, an equally important turning point leading to enhanced

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research in the aetiology and pathophysiology of obesity has come with the recognition of the condition as a chronic medical disease entity (World Health Organization, 1998) and the subsequent change in approach from many regulatory and reimbursement bodies. By characterising obesity as a disease, research and subsequent development aiming at obtaining approval of registration of drugs specific for the obesity indication became both more accepted and potentially rewarding. Like other chronic conditions, obesity will need to be controlled through continuous treatment to ensure permanent weight loss. Because of predicted long term medication of obesity as well as the moderate severity of condition, the regulatory requirements needed to be met by anti-obesity drugs are stringent, but even so this has not dampened the research commitments in the area. As evidenced from the many contributions to the current special issue of European Journal of Pharmacology, a large spectrum of therapeutic avenues towards weight reduction are possible. Peripheral targets include the signalling pathways regulating energy expenditure and lipid accumulation in adipocytes as well as compounds affecting absorption of nutrients from the gastrointestinal tract. Other peripherally acting pharmacotheraputic agents aim at mimicking or even enhancing the post-prandial absorptive phase thereby inducing premature satiety and decreased gastric motility. Centrally acting agents ideally influence specific satiety and hunger mediating pathways without impact on other behavioural modalities. However, central neural pathways integrate and activate both hedonic and energy-conserving aspects of drives towards eating. The term ponderostat (latin *pondus*: weight) is often used about the putative neural set point adjusting body mass by integrating multiple input reflecting changes in primarily energy availability but maybe also body weight per se. With an emerging concept of a ponderostat, it should be possible to identify and characterise its constituents and consequently to develop drugs, which activate various components of this circuit without affecting hedonic qualities of food and feeding.

2. Materials and methods

2.1. Body weight homeostasis

In 1879, Bernard first formulated the principle of homeostasis, arguing that ‘‘La fixté du milieu intérieur est la condition de la vie libre’’ (Bernard, 1879). Body mass is but one of several physiological parameters being regulated within relatively narrow limits, although the structural elements of such biological device are far from understood. The original adipostat hypothesis put forward by Gordon Kennedy claims that body weight is regulated based on a signal from adipose tissue acting together with short-term regulators of food intake to regulate feeding and overall energy balance (Kennedy, 1953). The adipostat theory has gained considerable evidence by the observation that body energy stores (i.e. adipocyte content of triacylglycerol) is tightly correlated to circulating levels of leptin and insulin, both of which impact neurones of the central nervous system, but our understanding of the ‘‘adipostat’’ is still far from complete. The most intriguing unanswered question is how the brain increases or decreases body-weight, that is, adjust the body-weight set-point. One of the most classical and clear-cut examples is the seasonal cycling of body-weight in hibernating species (Mrosovsky, 1990; Mrosovsky and Powley, 1977). The evolutionary drive to develop an adipostat has been a world characterised by scarcity. Thus, up to the twentieth century humans and beast alike have struggled to locate, obtain and cultivate enough calories to sustain survival. However, the rheostatic machinery of the body weight maintaining device is far from as simple as that of the body’s homeothermic thermostat. As evidenced by the epidemic rise in prevalence of obesity, human body weight is not well defended in a world of plenty and it seems reasonable to propose that endocrine signals from the energy storing adipocytes are biologically most meaningful as ‘‘go’’ signals to reproduction. Ideally, drugs alleviating the burden of obesity should enhance or return the otherwise sluggish upper reference set point level of the adipostat preferably by reducing energy intake and/or increase fatty acid beta-oxidation.

2.2. Components of an adipostat

Biological systems, which gauge the magnitude of a disturbance and activate qualitatively and quantitatively appropriate responses must have essentially the same functional component as those an engineer would introduce in his construction of a physical regulatory system. It should have one or even several sensors of the regulated variable. The sensor should convey online information about the regulated variable to a regulator, which upon receipt of a deflective signal should instigate one or more appropriate instructional signals activating the mediators of a correction response. The activity of these mediators obviously is to return the regulated variable towards a pre-set value. The set level of firmly controlled biological variables maybe subject to changes both upwards and downwards. Analogous to the engineered physical regulatory system mammalian rheostats are fitted with an optional regulation of the set point. General consensus has emerged to locate most of the components of the mammalian adipostat within the central nervous system, and thanks to the tremendous increase in neurobiological studies of energy homeostasis, it is now possible to locate and neurochemically phenotype many of the components of the adipostat. Fig. 1 is an outline of the hypothalamic adipostat as we propose it. The sensory components include leptin and insulin sensitive neurones of the hypothalamic arcuate and ventromedial nuclei and most likely also the nucleus of the solitary tract, which is also deeply engaged in the short term regulation of feeding

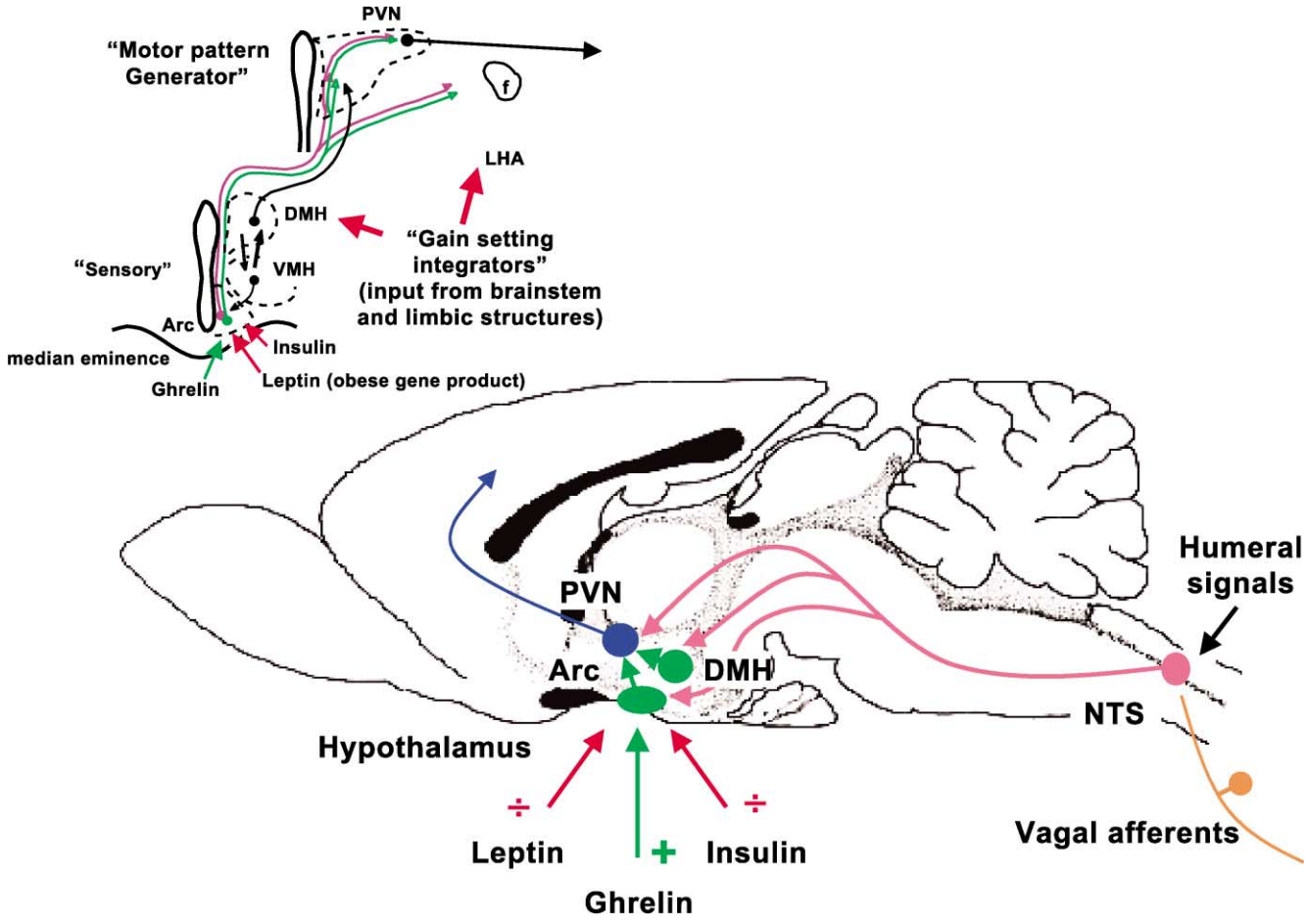

Fig. 1. Schematic drawing highlighting structures that possibly constitute the central adipostat. The sensory areas—the Arcuate nucleus (Arc) and the nucleus of the solitary tract (NTS)—are shown in blue. These areas located in close proximity to blood–brain barrier-free areas harbour neurones that are sensitive to circulating hormones (arcuate nucleus and nucleus of the solitary tract) as well as to neurally mediated meal related signals emanating from the gut (nucleus of the solitary tract). The areas feed into the hypothalamic motor pattern generator orchestrating the diverse endocrine, autonomic and behavioural physiological parameters associated with food intake and energy expenditure. The sensitivity of the hypothalamic paraventricular nucleus to sensory input is determined by a "set-point" regulator—possibly located in the dorsomedial hypothalamic nucleus (see text for details).

and energy expenditure. The regulator(s) of the adipostat consist of neurones in the hypothalamic paraventricular (PVN) and perifornical (PeF) nuclei. Obviously, other nuclei are integrated as down stream relays in the complex pathways connecting the motor pattern generators of the PVN and PeF to the effector neurones regulating such diverse actions as food seeking and energy expenditure. The anatomical localisation of neurones setting the gain and consequently generation of the adipostat set point is less well understood. Actually, it is still debatable if the adipostat system actively defends the system against an upward drift of the weight set level. As noted above, given the fact that only in the last 50 years has food-surplus been present in parts of the world and therefore no obvious survival advantage has been present from such a function during the development of mammalian species. Today, good candidate areas harbouring the gain setting neurones are the dorsomedial hypothalamic nucleus and the lateral hypothalamic area.

2.3. The sensor(s) of the adipostat

Shortly after the cloning of the leptin receptor, anatomical localisation of central neurones expressing the long signalling Ob–Rb form was clarified. Kinetic studies using radiolabelled leptin have clearly shown that leptin is transported across the blood–brain barrier via a saturable but not fully understood mechanism (Banks, 2001). Once plasma levels of leptin exceed 15 ng/ml, transport is saturated and intracerebroventricular levels increase no further, supporting the view that hyperleptinaemia has little impact on neurones shielded effectively by the blood–brain barrier (Unger, 2000). However, several areas of the brain including most of the circumventricular organs act as neurohaemal zones capable of sensing circulating hormones as well as releasing centrally produced neuroendocrine transmitters. According to the model depicted in Fig. 1, peripheral signals related to hunger, satiety and energy stores converge upon two primary sensory components of the adipostat, the arcuate

nucleus and the nucleus of the solitary tract. Leptin acts as such a feedback signal translating fat-volume into an endocrine signal and the long form of the leptin receptor, Ob–Rb, is expressed at high levels in neurones of both nuclei. The reflection by the blood–brain barrier of the median eminence and the area postrema is relative as evidenced by kinetic studies of neuropeptide entry from plasma to the brain (Whitcomb et al., 1990). Thus, neurones of the arcuate nucleus (adjacent to the median eminence) and the dorsal vagal complex are likely to be affected by circulating levels of leptin throughout the full range of plasma concentrations. In contrast, central areas fully shielded by the blood–brain barrier are probably less sensitive to upward excursions of plasma leptin as they would be exposed only to the highest possible intracerebroventricular concentration of leptin. The obvious conclusion from this interpretation would be that neurones of the arcuate nucleus and/or nucleus of the solitary tract are ideally suited to mediate anorectic and catabolic functions of leptin, whereas leptin receptors in other brain areas like the catecholaminergic neurones in the lateral hypothalamic area and the serotonergic neurones of the raphe system would mediate behavioural effects of leptin (Hay-Schmidt et al., 2001). Using rats exposed to neonatal monosodium glutamate lesioning of the arcuate nucleus, we have shown that the arcuate nucleus is essential in mediating the anorectic effect of leptin (Tang-Christensen et al., 1999b). In the arcuate nucleus, the Ob–Rb is expressed in both Neuropeptide Y/Agouti related peptide (NPY/AGRP) co-expressing neurones as well as in proopiomelanocortin/cocaine amphetamine regulated transcript neurones (POMC/CART) co-expressing neurones (Håkansson et al., 1998). The overall function of NPY/AGRP neurones is to stimulate an anabolic state whereas POMC/CART neurones induce catabolism (Schwartz et al., 2000). Recent electrophysiological studies have shown that leptin via an intricate neural network induces net-activation of POMC/CART neurones and net-inhibition of NPY/AGRP neurones (Cowley et al., 2001) confirming their candidacy as primary sensors of leptin. It is interesting that the distribution of Ob–Rb in the arcuate nucleus overlaps completely with that of insulin receptors and insulin receptor signalling protein-2 (IRS-2). Mice with site specific genomic deletion of the IRS-2 expression in the brain are overweight and restoration of IRS-2 expression in arcuate neurones normalises the obesity thereby confirming the anorectic and catabolic role of insulin in the central nervous system (Brüning et al., 2000). Even more interesting is the observation that intracellular signalling pathways of leptin and insulin converge on a final common pathway, the phosphatidyl inositol-3 (PI3)-kinase system (Niswender et al., 2001) which maybe subject to pharmacological manipulation.

Less is known about leptin sensitivity of neurones of nucleus of the solitary tract. Local administration of leptin into the fourth ventricle as well as systemic injections of leptin induce expression of c-Fos in ascending catecholaminergic/cholecystokinin (NA/CCK) as well as ascending glucagon-like peptide-1 and glucagon-like peptide-2 (GLP-1/GLP-2) neurones (Elias et al., 2000, own unpublished). Both of these populations of neurones express Ob–Rb suggesting that they are directly sensitive to leptin, but recent studies have shown that local application of leptin at gastric vagal afferents induces neuronal activity of neurones of the nucleus of the solitary tract (Yuan et al., 1999). These observations imply that brainstem neurones are affected by circulating leptin both via the neurohaemal zone of the area postrema and adjacent subpostreme area as well as via vagal afferents from the gastrointestinal tract. Despite ambiguous results obtained in clinical trials using recombinant human leptin (Heymsfield et al., 1999), it is possible that activity of satiety inducing neurones is augmented by the concomitant stimulation by leptin. Thus, intracerebroventricular administration of leptin prior to gastric distension of systemic cholecystokinin administration causes much more pronounced satiety than either of the stimuli alone (Emond et al., 2001; Matson and Ritter, 1999). The structural basis for such interaction is likely to be aforementioned ascending neurones of the nucleus of the solitary tract, because postprandial signals of systemic cholecystokinin, GLP-1, and gastric distension as well as leptin converge on these neurones (Fig. 2).

Other brain areas are sensitive to leptin. Because pharmacological agents, which in the CNS increase extracellular concentrations of serotonin have been studied and even exploited extensively as potential anorectic drugs, it is relevant to focus on possible interactions between leptin and the ascending raphe neurones. We and the others have shown that the major proportion of ascending raphe neurones express Ob–Rb, probably constituting the anatomical basis of leptin dependent serotonergic input to hypothalamic areas involved in regulation of feeding behaviour, and reproduction (Finn et al., 2001; Hay-Schmidt et al., 2001). It is evident that food stored as triacylglycerol in adipocytes constitute a long-term signal of its caloric value but food is also hedonically rewarding. It is likely that central neural pathways that respond to energy stores converge with neural circuits involved in motivation and reward. Support of such convergence comes from the recent discovery that leptin modulates neuronal activity in central areas involved in reward. The mesolimbic dopaminergic neurones have been shown to be involved in mediating several rewarding qualities of food. In our recent analysis of Ob–Rb localisation in central catecholaminergic neurones, less than 1% of the classic mesolimbic neurones found in the caudal portion of the A10 area (ventral tegmental area) expressed the functional leptin receptor (Hay-Schmidt et al., 2001). However, in the rostral portion of the dopaminergic A10 area, as well as in the A11, and A13 areas, a high degree of co-localisation was found. These limbic neurones are likely to be involved in rewarding aspects of feeding behaviour, because pharmacological blockade of dopaminergic D2-receptors in their targets areas including the lateral hypo-

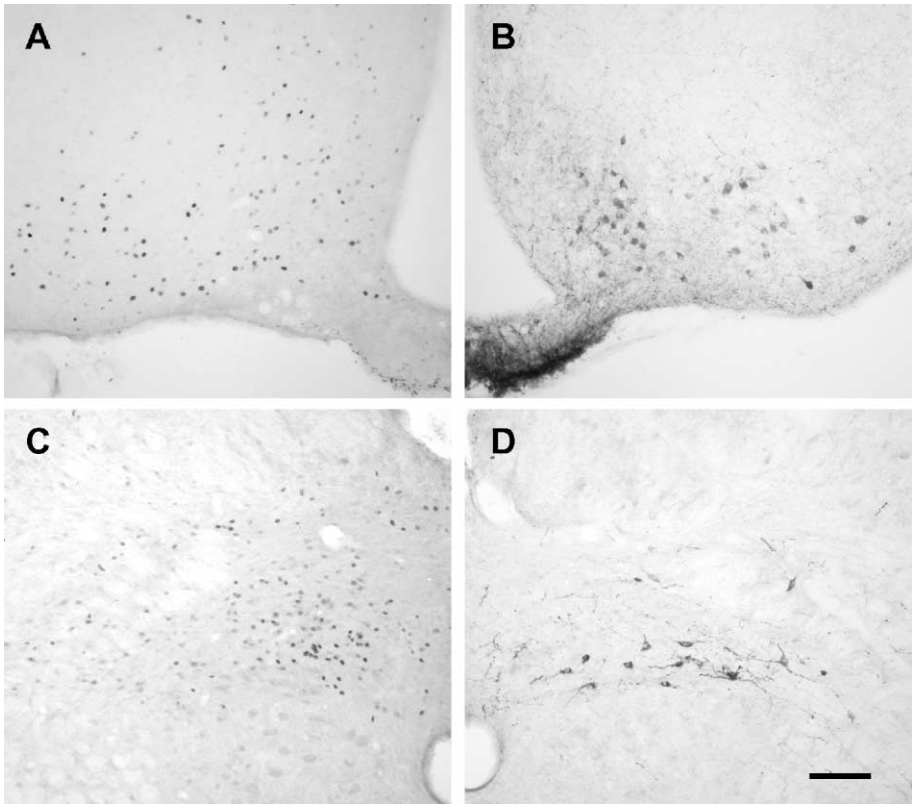

Fig. 2. Microphotographs of single sections from rat brain. (A) and (B) are images of the mid-part of the hypothalamic arcuate nucleus (Arc). (A) shows the pattern of c-Fos expression in the Arc 90 min after an i.v. injection of leptin (1.5 mg/kg). (B) shows the location of cocaine amphetamine regulated transcript-immunoreactive neurons in the arcuate nucleus (note that cocaine amphetamine regulated transcript-immunoreactivity and c-Fos-immunoreactivity in the Arc overlap). (C) and (D) show the caudal portion of the nucleus of the solitary tract and dorsal vagal complex. (C) shows the c-Fos response in the nucleus of the solitary tract following leptin i.v. and (D) the distribution of GLP-2 immunoreactive elements at the same level. Scale bar: 100 μm.

thalamus decrease intake of palatable substances. In a recent study, Figlewicz et al. (2001) have shown that leptin actually reverses sucrose-conditioned behaviours supporting the idea that leptin impacts on hedonic qualities of feeding. This observation calls for more studies of the behavioural aspects of the long-term signals mediating information about stored energy resources to the central nervous system.

2.4. The motor pattern generators

The hypothalamic paraventricular nucleus is recognised for its highly heterogeneous population of neurones serving diverse functional roles including neuroendocrine control of the anterior pituitary gland, release of systemically acting neuroendocrine transmitters via the posterior pituitary lobe, as well as control of autonomic output to most organs (Swanson, 1987). However, the PVN also influences animal behaviour associated with regulation of energy balance. Early lesion experiments have shown that the destruction of the hypothalamic paraventricular nucleus causes obesity and several studies using targeted intranuclear injections have shown that the hypothalamic paraventricular nucleus is a powerful mediator of several neuropeptides having an impact on feeding (Neuropeptide Y; Agouti related peptide; α-MSH; cocaine amphetamine regulated transcript peptide; Glucagon-like peptide-1). Following the analogy of an engineered homeostasis maintaining device, the hypothalamic paraventricular nucleus serves a role of a motor pattern generator responding to varied input from the sensory components of the adipostat (Fig. 1). At times with decreased energy stores, the net output of the hypothalamic paraventricular nucleus favours enhanced feeding and energy deposition by vagally induced insulin secretion. Despite years of intense research on the role of the hypothalamic paraventricular nucleus in feeding behaviour, the pathways emanating from the nucleus controlling this behaviour are still unknown.

2.4.1. Autonomic outflow

Most neurotransmitters influencing feeding behaviour also impact on metabolic rate such that inhibition of food intake goes hand in hand with increased energy expenditure. The effects of leptin on energy expenditure are mediated via enhanced sympathetic outflow as evidenced from rodent studies and our own experiments in non-human primates (Tang-Christensen et al., 1999a). The question arises whether identical or separate populations of neurones integrating circulating levels of leptin and insulin affect feeding behaviour and energy expenditure. Much evidence supports

the idea that a single group of sensory neurones activates parallel neuronal pathways. Thus, leptin induced activation of a-MSH neurones in the arcuate nucleus leads to a down stream activation of neurones involved in inhibition of feeding behaviour as well as in activation of neurones controlling energy expenditure and insulin secretion (Cone, 1999). The neurones mediating impact on feeding are largely unknown, whereas effects on energy expenditure are mediated via descending pathways synapsing on preganglionic autonomic outflow.

At present, it is unknown whether hypothalamic paraventricular nucleus neurones constitute the only relay between leptin sensitive neurones of the arcuate nucleus and preganglionic autonomic neurones but this possibility seems unlikely. Leptin induces c-Fos expression in POMC/CART neurones of the retrochiasmatic area and these neurones project directly to autonomic preganglionic neurones of the intermediolateral nucleus of the thoracic spinal cord (Elias et al., 1998). It is likely that this pathway in concert with descending fibres from the PVN and lateral hypothalamus mediate leptin's stimulatory impact on the sympathetic output. Leptin sensitive NPY/AGRP and POMC/CART neurones of the arcuate nucleus also innervate the diffuse portion of the dorsomedial hypothalamic nucleus as well as the rostral perifornical zone of the lateral hypothalamic area (Bagnol et al., 1999; Sawchenko et al., 1985). The lateral hypothalamus including the perifornical zone contain numerous neurones projecting to the autonomic preganglionic vagal motor nucleus and to a lesser degree to sympathetic preganglionic neurones of the intermediolateral cell column of the thoracic spinal cord (Buijs et al., 2001). Neuropeptide Y activates a relatively high proportion of descending neurones of the perifornical region having an impact on preganglionic neurones of the vagal motor nucleus supporting the suggestion that leptin sensitive arcuate neurones influence insulin secretion via a relay in the lateral hypothalamus (Vinuela and Larsen, 2001). Judging from the hypothalamic distribution of Agouti related peptide immunoreactive nerve terminals, the rostral portion of the perifornical zone most heavily innervated by the arcuate nucleus constitute an anatomical continuum of the lateral parvicellular part of the hypothalamic paraventricular nucleus. This area appears to coincide with the feeding inducing perifornical nucleus most powerfully activated by Neuropeptide Y (Stanley et al., 1985). Descending projections of the lateral parvicellular part of the hypothalamic paraventricular nucleus densely innervate both sympathetic and parasympathetic autonomic preganglionic neurones (Cechetto and Saper, 1988), and together with other descending hypothalamic paraventricular nucleus neurones and aforementioned lateral hypothalamic neurones they most likely constitute the motor pattern generators of the adipostat having an impact on energy expenditure.

These neurones are obvious targets for pharmacological treatment of obesity because activation inadvertently would increase energy expenditure. Both Neuropeptide Y and melanocortin receptors are expressed in neurones of the lateral hypothalamus, and ultrastructural studies have shown that NPY/AGRP as well as melanocortinergic nerve fibres make synaptic contact with hypocretin/orexinA positive neurones in the lateral hypothalamus. (Horvath et al., 1999). A high proportion of lateral hypothalamic neurones innervating the preganglionic autonomic outflow neurones express the appetite stimulating neurotransmitter melanin concentrating hormone (MCH) (Buijs et al., 2001).

2.4.2. Behavioural outflow

The exact localisation of Neuropetide Y and melanocortin sensitive neurones initiating or inhibiting feeding behaviour is unknown as is also their neurochemical phenotype. Given that so much time and effort has been put into solving the wiring diagram of central pathways constituting the adipostat, it is seems embarrassing that this riddle remains unsolved. In the hypothalamic paraventricular nucleus, neuropeptide Y targets at least four different receptors (Y1, Y2, Y4 and Y5). Continuous central infusion of Neuropeptide Y induces a metabolic syndrome characterised by hyperphagia, hyperinsulinaemia and overweight (Sainsbury et al., 1997). Also, the recently discovered endogenous growth hormone secretagogue, Ghrelin, which almost exclusively targets arcuate Neuropeptide Y neurones induces a syndrome of hyperphagia and obesity upon chronic administration (Tschöp et al., 2000). Obviously, these observations strongly support the idea that part of the sensory component of the adipostat, the arcuate Neuropeptide Y neurones, has an impact on central effector neurones inducing food intake. Neuropharmacological experiments, as well as evidence from targeted functional deletion by gene knock outs, suggest that at least Y1, Y2 and Y5 receptors are involved in mediating the orexigenic effects of Neuropeptide Y (Marsh et al., 1998; Naveilhan et al., 1999; Pedrazzini et al., 1998). The Neuropeptide Y knock-out mouse has fully compensated for the absence of the most potent orexigenic neuropeptide and respond normally to challenges of energy homeostasis (Erickson et al., 1996) and chronic administration of mixed Neuropeptide receptor Y antagonists has not been able to reliably reduce their body weight. This may partly explain why Neuropeptide Y receptor antagonist has failed to induce lasting anorexia and negative energy balance, but it is also possible that the endogenous tone of arcuate Neuropeptide Y neurones in well fed individuals is very low rendering pharmacological antagonism without functional impact.

In comparison to central Neuropeptide Y pathways, the neuronal pathways containing Pro-opiomelanocortin derived peptides are easier to comprehend primarily because forebrain Pro-opiomelanocortin nerve fibre all originate in the arcuate nucleus (Sawchenko et al., 1982). In the brainstem, a small population of Pro-opiomelanocortin containing neurones is found in the nucleus of the solitary tract. Postranslational processing of pro-opiomelanocortin gives rise to melanocortins including α-melanocyte stimulating hormone

(α-MSH), as well as β-endorphin. Due to their impact on feeding and energy expenditure, most emphasis has been put on melanocortinergic pathways, but it is worthy to mention that central administration of β-endorphin gives rise to increased food intake (Yim and Lowy, 1984). However, lessons learned from both MC3-R and MC4-R receptor knock out animal models as well as pro-opiomelanocortin knock out mice support the generally held view that functional activation of central melanocortinergic pathways favours negative energy balance through decreased feeding and enhanced energy expenditure (Butler et al., 2000; Huszar et al., 1997; Forbes et al., 2001). The pharmacological relevance of this has not been fully emphasised by chronic dosing experiments with α-MSH because tachyphylaxis develop in less than 2 days with resulting minor impact on cumulated food intake and body weight (McMinn et al., 2000). However, lasting effects on insulin and leptin was observed in rats subjected to chronic α-MSH infusion suggesting that the a-MSH loaded to the osmotic minipumps remained biologically active throughout the study (McMinn et al., 2000). Chronic central antagonism of the MC3-R and MC4-R receptors by (Ac-Nle^4-c[Asp^5, D-2′ Nal^7, Lys^{10}]α-MSH-4-10NH$_2$)^{10} (SHU9119) gives rise to a metabolic syndrome characterised by decreased energy expenditure and increased feeding, obesity and hyperinsulinaemia (Adage et al., 2001). The positive energy balance and hypothermia are maintained even in SHU9119 treated animals pair fed to vehicle treated animals confirming that endogenous melanocortins impose a constant stimulatory tone upon catabolic pathways (Adage et al., 2001).

The most obvious target neurones for both Neuropeptide Y and α-MSH are melanin concentrating hormone neurones of the perifornical nucleus and medial region of the lateral hypothalamus. Melanin concentrating hormones containing pathways appear to be attractive candidates for pharmacological manipulation by anorectic agents. Evidence obtained from transgenic mice models, either having the melanin concentrating hormone gene deleted or being over expressed, clearly shows that melanin concentrating hormone is an anabolic neuropeptide stimulating food intake and decreasing energy expenditure (Shimada et al., 1998; Qu et al., 1996; Ludwig et al., 2001). Melanin concentrating hormones containing neurones project broadly to numerous cerebral target areas including the prefrontal cortex as well as the hypothalamic paraventricular nucleus (Bittencourt et al., 1992), and these projections to limbic cortex have been argued to be responsible for motivated feeding induced by melanin concentrating hormone (Flier and Maratos-Flier, 1998). In the lateral hypothalamus, melanin concentrating hormone neurones co-express Ob–Rb and it is possible that these neurones upon leptin activation reduce food-seeking behaviour. So far, targeted microinjections have been performed only at hypothalamic sites, of which the hypothalamic paraventricular nucleus appears to be the most sensitive in mediating the orexigenic actions of melanin concentrating hormone (Rossi et al., 1999).

2.5. Gain setting devices

It is often discussed whether the adipostat is equipped with a device enabling upwards or downwards regulation of the gain of the system of the so-called set point. Experimental evidence favouring the presence of specific set point generator has come from selective breeding experiments of Sprague–Dawley rats (Levin et al., 1997). After breeding for several generations, a subgroup of animals developed a clear susceptibility to develop diet-induced obesity and impaired glucose tolerance when exposed to high-energy diet. Conversely, another group of animals remains lean despite exposure to high-energy diet (diet-resistant). However, both diet-induced obese and diet-resistant animals are fully capable of defending their mean body weights by decreasing or increasing food intake in response to periods of overfeeding and food restriction, respectively (Levin and Keesey, 1998).

Our knowledge of the anatomical location of gain setting neurones is very limited, but the dorsomedial hypothalamic nucleus constitute a possible anatomical site for an organismic body weight set point generating device (Fig. 1). Lesions of the dorsomedial hypothalamic nucleus render rats hypophagic and with resulting smaller body mass and size, but sham-lesioned animals pair-fed to the dorsomedial nucleus-lesioned become even smaller and display poorer feed efficiency than dorsomedial nucleus-lesioned animals supporting that neurones of the dorsomedial nucleus contribute positively to set the gain of the adipostat (Bernardis et al., 1988). In other words, the neurones of the dorsomedial nucleus render animals less capable of coping with situations characterised by reduced caloric intake. Similar, but less obvious and oppositely directed results have been obtained by lesioning neurones of the lateral hypothalamic area (Bernardis and Bellinger, 1996). Obviously, much more research is needed in this area, but it is tempting to speculate that a fully comprehensive insight to the neurochemistry of the gain setting devices would produce pharmacological agents capable of either up or down regulating body weight set points.

3. Cocaine amphetamine regulated transcript (CART): co-transmitter with pro-opiomelanocortin derived peptides in leptin sensitive neurones of the arcuate nucleus

Since our discovery that cocaine amphetamine regulated transcript expression in arcuate pro-opiomelanocortin neurones is dependent on leptin, many studies have been conducted to further elucidate the anorectic mode of action of the biologically active form, CART42-89. Originally, a part of the CART peptide was identified in hypothalamic extracts prepared for identification of hypothalamic releasing factors having an impact on anterior pituitary hormone release (Spiess et al., 1981). This observation was not

followed up until several years later when Douglass et al. (1995) identified a transcript in the rat forebrain using a polymerase chain reaction differential display technique to identify transcripts regulated by cocaine and amphetamine. The pharmacological exploitation of cocaine amphetamine regulated transcript peptide fragments in anti-obesity therapy has been somewhat stigmatised by the assumption that it constitute a down-stream mediator of addictive drugs cocaine and amphetamine. However, in our own efforts to identify forebrain areas in which cocaine amphetamine regulated transcript mRNA is regulated by increased dopaminergic transmission, we have been unable to verify any impact of amphetamine upon cocaine amphetamine regulated transcript mRNA in the accumbens and striatum. In order to obtain optimal anatomical resolution, we have used in situ hybridisation histochemistry in contrast to tissue extraction based polymerase chain reaction, but it seems unlikely that methodological differences can explain the apparent discrepancy between our recent observation and those reported by Douglass et al. (1995) (Vrang and Larsen, in preparation). Other researchers have also been unable to stimulate cocaine amphetamine regulated transcript expression in the nucleus accumbens by cocaine or amphetamine (Hurd et al., 1999). Thus, we suggest the scientific community to put time and effort in changing the essence of the CART acronym to a functionally more meaningful reflection of its diverse biological actions.

Using a polymerase chain reaction subtraction cloning, Sutcliffe et al. identified the cocaine amphetamine regulated transcript gene as the third most abundantly expressed in the rat hypothalamus (Gautvik et al., 1996). In the hypothalamus, cocaine amphetamine regulated transcript is co-localised with several neurotransmitters involved in regulation of feeding behaviour. Thus, cocaine amphetamine regulated transcript co-localises with pro-opiomelanocortin derived peptides in the arcuate nucleus, and with melanin concentrating hormone in the lateral hypothalamus. In the arcuate nucleus, cocaine amphetamine regulated transcript mRNA levels are regulated by leptin, and central administration of C-terminal cocaine amphetamine regulated transcript peptides inhibits feeding (Kristensen et al., 1998). Until now, the most compelling physiological evidence supporting a role of cocaine amphetamine regulated transcript as an endogenous satiety factor has come from immunoneutralisation experiments showing that central administration of anti-cocaine amphetamine regulated transcript antibodies increases overnight food intake in rats (Kristensen et al., 1998; Lambert et al., 1998). A long term role in energy homeostasis also seems plausible because cocaine amphetamine regulated transcript expression in pro-opiomelanocortin neurones of the arcuate nucleus is negatively regulated by circulating leptin (Kristensen et al., 1998). In a subchronic experiment infusing either 4.8 or 12 μg/day/rat into the lateral ventricle for 7 days, we have shown that adult male rats decrease their food intake and display dose-dependent loss of body weight compared to vehicle infused animals over the initial 4–5 days of treatment (Larsen et al., 2000b). However, as seen with central infusions of α-MSH (but not the mixed MC3-R/MC4-R receptor agonist MT-II) tachyphylaxis develop and the catabolic effects of CART-(42–89) become only transient. At the end of the chronic infusion experiment, full biological activity was found for CART-(42–89) remaining in the osmotic minipumps. In this chronic infusion experiments as in acute injections, a dose-dependent motor disturbance was also seen. Animals receiving an intracerebroventricular dose of CART-(42–89) into either of the ventricles display unsteady gait which is exaggerated upon voluntary movement with clear parallels to trunkal ataxia induced by central cerebellar lesions. Also, effects on the motor programming underlying licking behaviour have been demonstrated to be modified by cocaine amphetamine regulated transcript peptides (Aja et al., 2001) and cocaine amphetamine regulated transcript peptides injected into the ventral tegmental area have been shown to increase locomotor behavior by a dopamine D2 receptor dependent mechanism (Kimmel et al., 2000), pointing to widespread and very complex roles of cocaine amphetamine regulated transcript peptides in motor control/planning. Recently, a number of papers examining local injections of cocaine amphetamine regulated transcript peptides into hypothalamic nuclei have emerged. Notably, these motor disturbances have not been seen in animals receiving intraparenchymal injections aiming at individual hypothalamic nuclei. Wang et al. (2000) observed profound anorexia upon injection of rather large doses of CART-(42-89) directly into the hypothalamic paraventricular nucleus, and in a recent study, Abbott et al. (2001) have observed a paradoxical increase of food intake after administration of 0.04 nmol CART-(42-89) into several hypothalamic nuclei. Although the feeding assay employed by Abbott et al. (2001) is not directly comparable to that used in other studies of CART induced anorexia, it is worthy of mention that they observed the well known anorectic action of the peptide after intracerebroventricular injection of CART-(42–89). At present, it is unknown whether their locally induced orexigenic action is a specific reflection of an anabolic action of cocaine amphetamine regulated transcript or whether it reflects non-specific stimulation of mastication as seen for example in response to noradrenaline injections into the hypothalamic paraventricular nucleus. Another possibility is that CART-(42–89) in some nuclei elicits stress-induced feeding responses, because it is well known that central CART injections activate the hypothalamo-pituitary adrenocortical axis, presumably by a direct action on the cortricotrophin-releasing hormone containing neurones of the hypothalamic paraventricular nucleus (Vrang et al., 2000). However, an overall catabolic role of cocaine amphetamine regulated transcript peptides is also supported by the recent observation that central administration of the peptide inhibits fasting induced suppression of paraventricular expression of thyreotrophin-releasing hormone (Fekete et al., 2000). Central administration of CART-(42–89) has

also been shown to halt gastric acid secretion as well as gastric motility (Okumura et al., 2000). The anatomical pathways mediating this action are likely to involve descending hypothalamic neurones targeting autonomic outflow to the gastric portion of the gastrointestinal tract.

When offered a high caloric diet, animals engineered to a life without cocaine amphetamine-regulated transcript peptides showed significant increases in weekly food consumption, body weight, and fat mass compared to wild-type littermates (Asnicar et al., 2001). However, cocaine amphetamine regulated transcript null mice are less sensitive to pain and given the relatively dense innervation of the dorsal horn lamina 1 and 3 by cocaine amphetamine regulated transcript immunoreactive fibres, it seems likely that cocaine amphetamine regulated transcript expressed in neurones of the spinal dorsal root ganglia are involved in neurotransmission of pain (Ohsawa et al., 2000). In the hypothalamus, CART-(42–89) is probably also involved in regulation of gonadotrophin-releasing hormone release as local application of the peptide to in vitro explant preparations of the mediobasal hypothalamus stimulates pulsatile gonadotrophin-releasing hormone release (Lebrethon et al., 2000). Evidence obtained from studies using Zucker (fa/fa) rats have shown that the stimulatory effect of cocaine amphetamine regulated transcript peptides upon gonadotrophin-releasing hormone release is downstream to leptin suggesting that cocaine amphetamine regulated transcript constitute the endogenous mediator of leptin's permissive effects on reproduction.

Two silent polymorphisms of the 3′ untranslated region of human cocaine amphetamine regulated transcript have been known for a while but during a recent screen of Italians suffering from early-onset obesity a missense mutation of G729C resulting in a substitution of Leu with Phe at codon 34 was discovered in a 10-year-old obese boy (del Giudice et al., 2001). Subsequent analysis of the subject's family showed highly significant co-segregation of the mutation with severe obesity and decreased metabolic rate (del Giudice et al., 2001). Several questions regarding the CART phenotype of the probands carrying the mutation remains to be answered, but the N-terminal changes of the pro-peptide emphasise the importance of a thorough characterisation of this part of the cocaine amphetamine regulated transcript. So far only the C-terminal parts of the cocaine amphetamine regulated transcript peptide has been subjected to chromatographic studies aiming at identifying postranslational processing products of cocaine amphetamine regulated transcript (Thim et al., 1999). Thus, it is possible that N-terminal fragments of the cocaine amphetamine regulated transcript peptide precursor are present as biologically active fragments within the central nervous system and to our knowledge comprehensive studies aiming at testing this possibility are yet to be conducted.

The highly heterogeneous pattern of cocaine amphetamine regulated transcript synthesising neurones in central nervous systems constitutes a tremendous challenge to an isolated pharmacological exploitation of its actions on energy homeostasis. However, because cocaine amphetamine regulated transcript peptides are co-localised with melanocortins in the leptin sensitive neurones of the arcuate nucleus, future pharmacological endeavours should concentrate on studies employing cocktails of the subthreshold doses of the biologically active peptide fragment released from these neurones.

4. Glucagon-like peptides 1 and 2 (GLP-1/GLP-2): co-transmitters in ascending leptin sensitive neurones targeting hypothalamic nuclei involved in setting the gain of the adipostat

In neurones of the nucleus of the solitary tract as well as in enteroendocrine L-cells of the small ileum and colon, postranslational processing of preproglucagon gives rise to equimolar amounts of glucagon-like peptides 1 and 2 (Larsen et al., 1997). Practically, no glucagon is made in these regions. We have studied both peripheral and central GLP-1 and GLP-2 containing systems extensively, and we have found that GLP-1 in the central nervous system as well as in the periphery induces satiety but via different modes of action (Tang-Christensen et al., 1996; Larsen et al., 2001). Our immunohistochemical analysis of central GLP-1/GLP-2 containing pathways confirms a complete overlap of distribution suggesting complete co-localisation. Whereas GLP-1 receptors are expressed in several hypothalamic nuclei, including, the paraventricular, arcuate and dorsomedial nuclei, GLP-2 receptors are expressed exclusively in neurones of the compact region of the dorsomedial nucleus (Tang-Christensen et al., 2000). Highest densities of GLP-1/GLP-2 fibres are seen in the hypothalamic paraventricular nucleus and the ventral diffuse portion of the hypothalamic dorsomedial nucleus, where the dendritic aborisation of neurones of the compact portion of the dorsomedial nucleus prevail. Peripheral administration of leptin induces c-Fos expression in ascending GLP-1/GLP-2 containing neurones of the nucleus of the solitary tract (Elias et al., 2000) and central administration of leptin into the fourth ventricle induces expression of preproglucagon mRNA in the nucleus of the solitary tract (Goldstone et al., 2000). In a recent study, we have shown that the hypothalamic content of GLP-1 and GLP-2 is inversely correlated to the energy balance reflecting that synaptic release of GLP-1/GLP-2 is increased during states of voluntary overfeeding (Tang-Christensen et al., 2001). This observation strongly supports that central preproglucagon derived peptides serve roles as satiety factors.

Several pharmacological studies have focused on the role of GLP-1 as an endogenous satiety factor but interpretations have been hampered by interference of exogenous GLP-1 with central targets involved in other behavioural modalities. Considering the wide spread distribution of GLP-1 receptors in the central nervous system, it is hardly surprising that several targets is influenced by intracerebroventric-

ular administration of GLP-1 and analogues hereof. In a pharmacological context, the relevant question to address is whether centrally acting GLP-1 agonists are candidate anorectic agents with none or only few tolerable side effects. Generally, induction of aversive behaviour is considered an indicator of drug-induced anhedonia and consequently an indicator of potential drug induced malaise. As many other neuropeptides involved in regulation of energy homeostasis, central administration of high doses (10 μg) of GLP-1 induces taste aversion (Tang-Christensen et al., 1998). However, site directed micro-injections of GLP-1 into the hypothalamic paraventricular nucleus induces pharmacologically specific inhibition of feeding without induction of taste aversive behaviour (McMahon and Wellman, 1998). In animals having their arcuate nucleus lesioned by neonatal monosodium glutamate treatment, central administration of 10 μg GLP-1 has lost its anorectic potential but is still inducing taste aversion (Tang-Christensen et al., 1998). Further support of dissociated specific satiety inducing central targets of GLP-1 and non-specific taste aversion inducing central targets come from lesion studies by van Dijk and Thiele (1999) showing that the hypothalamic paraventricular nucleus constitutes a target where GLP-1 elicits satiety whereas the central amygdala and the parabrachial nuclei constitute areas involved in mediating GLP-1 induced taste aversion. Also, chronic repetitive central administration of the GLP-1 receptor antagonist, exendin-9–39, enhances food intake suggesting that an endogenous tone of satiety mediating GLP-1 exists in central pathways mediating body weight homeostasis (Meeran et al., 1999).

Given the apparent complete co-existence of GLP-1 and GLP-2 in ascending neurones, we speculated that GLP-2 may also constitute a central mediator of satiety. To our surprise, in situ hybridisation histochemistry showed that in adult rats, GLP-2 receptor expression is almost exclusively present in neurones of the compact part of the dorsomedial hypothalamic nucleus (Tang-Christensen et al., 2000). This region of the dorsmodial nucleus contains a substantial number of Neuropeptide Y mRNA expressing neurones, but so far immunohistochemical analyses have failed to reliably demonstrate presence of neuropeptide Y in perikarya and neurites originating from these neurones. Thus, the neurochemical phenotype of GLP-2 receptor expressing neurones is still uncertain but given their localisation they are likely to constitute part of the dorsomedial nucleus gain setting entity involved in body weight homeostasis. Central administration of GLP-2 induces premature satiety to freely fed rats as well as inhibition of both starvation and Neuropeptide Y induced feeding (Fig. 3). Glucagon-like peptide-2 actions on feeding are dependent on intact central GLP-1 receptors because pharmacological antagonism of GLP-1 receptors by prior administration of exendin-9–39 abolishes GLP-2 induced anorexia (Tang-Christensen et al., 2000). The most likely interpretation of this finding is that GLP-1 and GLP-2 receptors act in parallel requiring both to be fully operational in order to induce anorexia. Observations from

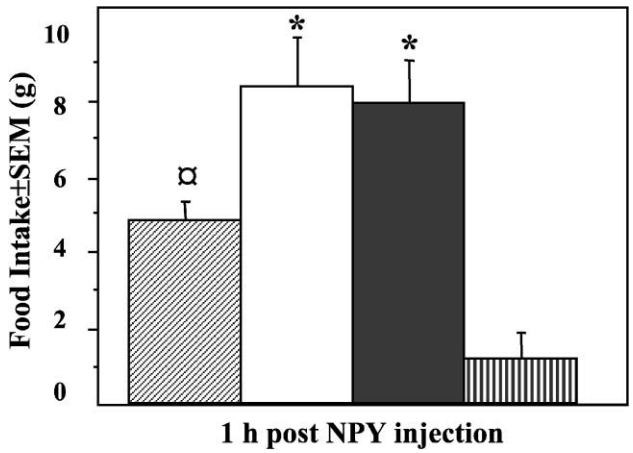

Fig. 3. Glucagon-like peptide-2 abolishes Neuropeptide Y induced feeding. Central administration of 10 μg GLP-2 prior to the administration of Neuropeptide Y (5 μg) leads to a significant inhibition of Neuropeptide Y induced feeding (hatched bar) when compared to vehicle (white bar). Prior administration of the inactive N-truncated GLP-2 analogue — GLP-2(5–33) did not alter Neuropeptide Y induced food intake (grey bar). Two consecutive injections of vehicle had no effect on feeding behaviour. $N = 8–14$, * $p < 0.05$ vs. vehicle/vehicle; ¤ $p < 0.05$ vs. vehicle/NPY or vehicle/vehicle ANOVA followed by Fisher post hoc analysis.

mice have given somewhat different results, because prior central administration of exendin-9–39 potentiates the anorectic actions of central GLP-2. The reason for this discrepancy is at present unknown, but our own in vitro experiments on transfected cell systems expressing human GLP-2 receptors clearly show that exendin-9–39 has virtually no affinity to the GLP-2 receptor (Fig. 4). Because of topographically very limited expression of central GLP-2 receptors, they constitute a pharmacologically very interesting target. Ideally, centrally acting GLP-2 agonists could induce satiety via interference with components of the body-weight set-point generating neuronal circuit. However, continuous administration studies are needed to confirm the lasting weight reduction by such treatment. Transgenic mice carrying a construct where a 1.5-kilobase fragment of the mouse GLP-2 receptor promoter directs LacZ expression show central expression of the construct in many brain regions (Lovshin et al., 2001). However, it is worthy to mention that lessons learned from transgenic mice carrying a LacZ construct driven by the α_{2A}-adrenoceptor showed expression of LacZ in numerous central brain regions not normally expressing α_{2A}-adrenoceptors and lack of LacZ expression in other areas known to express high levels of the endogenous α_{2A}-adrenoceptor (Wang et al., 1996). The fact that both ectopic expression and absence of appropriate expression of the transgene is seen it is of utmost importance to validate LacZ expression as a reliable reflection of endogenous GLP-2 receptor expression in the GLP-2/LacZ chimeras.

In addition to central GLP-1/GLP-2 targets, peripheral GLP-1 receptors are readily available for pharmacological exploitation. In the periphery, GLP-1 released from the L-

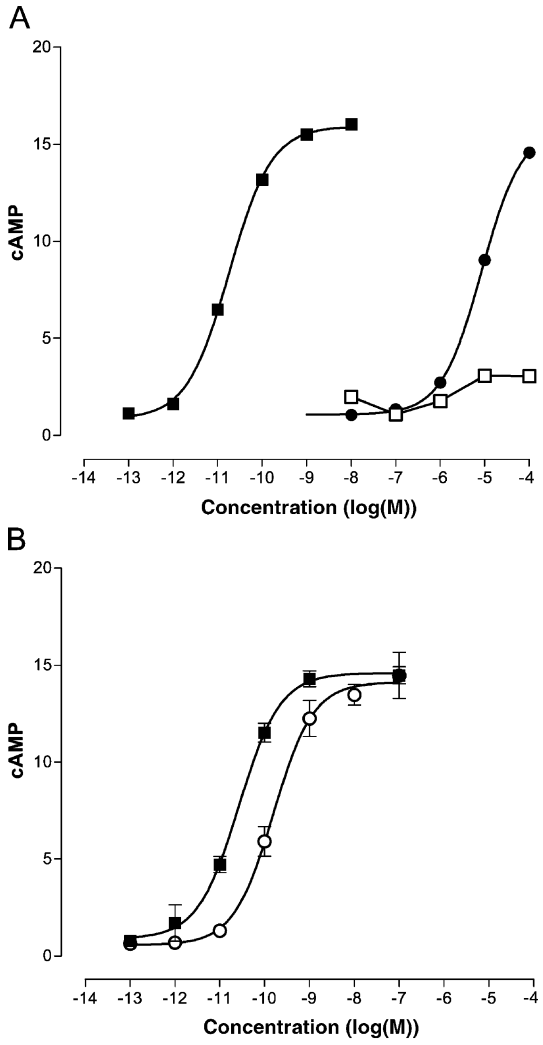

Fig. 4. GLP-1, exendin-4 and exendin-(9–39) interacted with the human GLP-2 receptor in baby hamster kidney cells with very low potency. (A) GLP-2 (filled squares), EC_{50} = 35.7 ± 13.7 pM (n = 8). GLP-1 was a full agonist with EC_{50} = 8,700,000 ± 0.21 pM (filled circles; n = 2) and exendin-4 was a very weak partial agonist (open squares; n = 2). (B) In agreement with the very low potency partial agonism of exendin-4 and low potency of GLP-1, only 10,000,000 pM of exendin-(9–39) was able to shift the GLP-2 dose–response curve to the right (open circles; n = 2). Data shown are from one representative experiment with duplicate samples, mean ± S.D. shown. The human GLP-2 receptor was cloned by PCR on cDNA from small intestine, inserted into pcDNA3.1 and stably transfected into baby hamster kidney cells using FuGene and selecting for Neomycin resistance. Clones expressing GLP-2 receptor were screened for their ability to generate a cAMP response after exposure to GLP-2. A functional GLP-2 receptor assay was established using the Flashplate technology (NEN).

cells is an incretin that stimulate insulin secretion and has a wide spectrum of other effects on pancreatic β-cells, and also inhibits glucagon secretion. However, most importantly GLP-1 also acts as an ileal brake reducing gastric emptying thereby reducing nutrient delivery rate to absorptive portions of the GI tract (Nauck, 1999). In both humans and rats, intravenous administration of synthetic GLP-1 acutely reduces food intake (Gutzwiller et al., 1999; Larsen et al., 2001). Promises of pharmacological use of GLP-1 and analogues hereof as anorectic agents have come from human and rodent studies. Rats carrying a GLP-1 producing glucagonoma become anorectic coincident with emergence of detectable plasma levels of biologically active GLP-1(7-36NH_2) (own observations, PBJ). Using the long-acting GLP-1 derivative, gamma-L-Glutamoyl(*N*-epsilon-hexadecanoyl)-Lys26,Arg34-GLP-1(7–37), also known as NN2211(Knudsen et al., 2000), we have shown that acute as well as twice daily administration for 10 days reduces food intake in rats with resultant decrease in body weight and fat content (Larsen et al., 2000a). NN2211 is designed for once daily administration in man, and is currently in phase 2 clinical development (Jakobsen et al., 2001). In a recent human study, continuous infusion of GLP-1 (4.8 pmol/kg/min) to type 2 diabetic patients gave rise to marked improvement of glycaemic control and caused moderate yet non-significant weight loss (Zander et al., 2001). There is reason to believe that future studies employing slightly higher doses, larger patient populations, and longer administration periods will prove GLP-1 analogues as efficient weight reducing agents, because subjective sensations of hunger and satiety remained affected even after several weeks of administration (Zander et al., 2001). The site of the anorectic action of peripherally administered GLP-1 is unknown but participation of both central and peripheral sites in GLP-1 induced anorexia are likely, because a recent study has shown that radiolabelled GLP-1 readily gains access to the central nervous system (Hassan et al., 1999). The nucleus of the solitary tract is situated adjacent to the blood–brain barrier free area postrema, and other studies using radiolabelled neuropeptides have shown that peripheral administration of neuropeptides gain access both to the area postrema as well as the adjacent subpostreme regions including the dorsal vagal complex (Whitcomb et al., 1990). Thus, it is likely that peripherally administered GLP-1 enters the nucleus of the solitary tract with resulting impact on ascending neurones involved in regulation of food intake. Interaction of GLP-1 with vagal afferents from the gastrointestional tract should also be considered as mediator of its anorectic actions because transection of the vagus nerve renders the stomach of anaesthetised pigs insensitive to the akinetic actions of intravenously administered GLP-1 (Wettergren et al., 1998). Probably both vagal afferents and GLP-1 receptors accessible from the periphery are responsible for the anorexia induced by GLP-1, because we have seen that bilateral subdiaphragmatic vagotomy on rats carrying the anorectic GLP-1 producing tumour has no impact on the development of anorexia (Jensen et al., 1998). Experience from human studies shows that the therapeutic window of GLP-1 infusions is limited because infusion doses above 5–6 pmol/kg/min induces nausea and vomiting, probably due to prolonged effects on gastric emptying and activation of emesis mediating pathways having their sensory components in the area postrema (Larsen et al., 1996). Considering the favourable effects on both glucose homeostasis and appetite, GLP-1 agonists

seem obvious candidates for pharmacological treatment of the overweight type 2 diabetes patient improving both glycaemic control and body weight with minimal risk of inducing reactive hypoglycaemia.

5. Future directions

Over the last decade, a structural outlay of the adipostat has emerged. In particular, several studies have focused on the structural and pharmacological organisation of the hypothalamic sensory components of the system, whilst our knowledge of functional and structural organisation of other sensory areas of the adipostat like the dorsal vagal complex is still very limited. The motor pattern generators affecting autonomic outflow are also well characterised as hypothalamic descending neurones of the hypothalamic paraventricular nucleus and lateral hypothalamic area. However, virtually nothing is known about the location and neurochemical phenotype of the motor pattern generators mediating the effects of leptin upon feeding. Finally, the gain setting neurones, so far only theoretically defined have yet to be identified. Intuitively, observations made from selective hypothalamic lesions suggest that neurones of both the dorsomedial hypothalamic nucleus and the lateral hypothalamic area serve functions as gain setting devices. Both cocaine amphetamine regulated transcript peptides and preproglucagon derived peptides could serve action as transmitters in these gain setting devices, but a tremendous amount of work lies ahead of before conclusive evidence about this is available.

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