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Mini-Review

Animal models of postinfectious obesity: Hypothesis and review

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In a recent review of obesity as a medical problem, it was stated that obesity is so common on a worldwide scale as to replace undernutrition and infectious diseases as the most significant contribution to ill health (Kopelman, 2000). As noted in a recent editorial, the probable cause of most cases of obesity in humans is thought to be a combination of genetic susceptibility and environmental factors (Rosenbaum and Leibel, 1999). A relatively small number of human cases reported to date are monogenic in origin; these cases represent counterparts of the classical monogenic obesities of rodents (Barsh *et al*, 2000). The possibility that virus infections might play a role in human obesity development has received little attention despite evidence that viruses can induce obesity in experimental animals. Recent progress relating to mechanisms by which virus infection may lead to obesity development is reviewed, with special reference to neurological infection with canine distemper virus (CDV).

To date, viruses from four different taxonomic groups as well as certain strains of the scrapie agent (Carp *et al*, 1998) have been shown to cause obesity in experimental animals. The diverse group of conventional viruses include the avian retrovirus (RAV-7) (Carter *et al*, 1983b), a human group D adenovirus, as well as a chicken adenovirus (Dhurandhar *et al*, 2000, 1992, respectively), strains of Borna disease virus (Narayan *et al*, 1983), and the morbillivirus, canine distemper virus (CDV).

We originally described the unexpected development of morbid obesity in outbred Swiss mice weeks to months after neurological infection with mouse-adapted CDV (Lyons *et al*, 1982), findings that were independently confirmed by others (Bernard *et al*, 1983). The pathogenic outcome depended on the age of the animals at the time of infection, as obesity developed only among animals infected as weanlings (4–6 weeks old); older animals seemed refractory to infection, yet younger animals uniformly succumbed to encephalitis. A range of pleiotropic effects of obesity were found for the postinfectious obese mouse including hyperphagia, impaired fertility, hypothermia, hyperglycemia, hyperinsulinemia, and hypercellular adipose tissue, (Lyons *et al*, 1982, and unpublished observations) that, when compared to the classic genetic models as well as hypothalamic lesion-induced models (Bray and York, 1979), showed remarkable congruence (Table 1). This pointed to the hypothalamus as vulnerable to the tropic effects of the virus, a fact borne out by a subsequent histopathological study (Nagashima *et al*, 1992).

In this study, CDV-infected weanling mice exhibiting mild signs of encephalitis were sacrificed 10 days postinfection, and evaluated by immunohistochemical staining. Clear evidence of CDV antigens in neuronal perikarya and processes, predominantly in peri-third ventricular hypothalamic areas, was observed, with the highest concentration of such CDV-reactive cells located in the arcuate-ventromedial area. In contrast, CDV antigen-positive cells were not found in obese brain specimens. To account for this, it was thought likely that antigen-expressing cells were either progressively lost in the postinfectious period due to viral cytopathic effects and/or, in the event of viral genome persistence, structural gene expression was suppressed. Also absent from obese specimens were inflammatory cell infiltrates, which were notable in the acutely infected specimens. Attempts to isolate infectious virus by cocultivation with susceptible VERO cells were unsuccessful.

Serial coronal sections from obese brain specimens and lean control littermates were stained with

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Table 1 Obesity-related phenotypes observed in the CDV postinfectious model compared to those reported for some established rodent models of obesity

Feature	*Hypothalamic lesioning: Knife cut, gold thioglucose etc. (no genetic basis)	*Genetically transmitted obesities				**CDV-induced obesity (no known genetic basis)
		ob/ob (recessive traits)	*db/db* (recessive traits)	*fa/fa*	*NZO* (polygenic)	
Obesity	++	+++	+++	+++	++	+++
Hyperphagia	+	++	++	++	+	+
Hyperglycemia	−	+++	+++	−	±	+
Hyperinsulinemia	+	++	+	++	+	++
Insulin resistance	±	+++	+++	++	+	++
Hypercellular adipose tissue	−	++	±	++	+	++
Hypothermia	−	++	++	−	ND	++
Impaired fertility	±	+++	+++	+++	?	+++
Reduction in brain weight	−	+	+	+	ND	+

+++ severe.
++ moderate.
ND: not determined.
± variable.
− absent.
*Data from Bray and York (Bray GA, York DA. 1979. *Physiol Revs* **59:** 719–809).
**Lyons, Faust, Hemmes, *et al.*, (1982); Buskirk, Lyons, Zabriskie (unpublished findings).

Kluver-Barrera (K-B) stain to permit the observation of cell soma and anatomic nuclei, while contiguous sections were immunostained for glial fibrillar acidic protein (GFAP) (to indicate astrocytosis and, indirectly, neuronal loss). This defined a diffuse pattern of gliosis which indicated a loss of neurons in hypothalamic areas involving the paraventricular nucleus (PVN) and dorsomedial area extending to the ventromedial and arcuate areas. This lesion approximated topographically the zone of viral antigen expression seen in the acutely infected weanling animals described previously. K-B-stained sections from one massively obese animal showed a severe depletion of cell soma from the PVN.

Obesity development was found to be associated with damage to two separate neuronal populations in the arcuate area. There was a significant loss of tyrosine hydroxylase (TH) (dopaminergic)-immunoreactive neurons in the arcuate area and their terminals in the median eminance. This was supported by microchemical analysis of dopamine levels in four hypothalamic areas sampled by the micropunch technique (Palcovits, 1973), including the preoptic area, the ventromedial nucleus, the dorsal area, and the arcuate/median eminence. Only in the latter was a highly significant drop in DA levels observed (MJL, unpublished observation).

Evidence of damage to a separate cell population of the area, namely pro-opiomelanocortin (POMC) neurons, was indicated by in situ hybridization studies that showed that POMC mRNA transcription was markedly reduced in obese specimens compared to lean littermate controls.

A recent study of CDV-induced obesity in mice (Bernard *et al*, 1999) confirmed the vulnerability of neurons of the medio-basal hypothalamus to virus infection and also the progressive loss of viral antigen expression with time. In some obese brain specimens, viral genomic sequences were shown to persist, and the possible role of such in inducing cellular dysfunction was discussed. Leptin receptor levels were measured in different areas of obese and control brains; receptor levels were found to be selectively reduced in hypothalamic nuclei (arcuate, dorsomedial, ventromedial) of the obese specimens only. Other brain areas of the obese specimens such as cortical areas were unchanged or showed elevated levels. It may be noted that, normally, the highest level of leptin binding in the brain parenchyma is found in the structures surrounding the median eminence, in the arcuate nucleus and parts of the dorsomedial and ventromedial nuclei (see the review by Elmquist *et al*, 1999), precisely in areas where electrolytic lesions were found to cause obesity in the classical experiments of Heatherington and Ranson (1940).

Infection of 10-day-old chicken embryos with RAV-7 resulted in a disease characterized by stunting of growth, hyperlipidemia, and obesity (Carter *et al*, 1983b). The major metabolic change observed was a decrease in thyroid hormone levels and administration of exogenous thyroxine was shown to prevent most of the disease symptoms. RAV-7-induced hypothyroidism (possibly centrally mediated) was stated as leading to secondary changes in lipid and energy metabolism such as reduced thermogenesis, which ultimately resulted in the obesity syndrome (Carter *et al*, 1983a).

A group of human obese subjects was screened for serum antibodies against the avian adenovirus SMAM-1, which had been shown to induce a unique obesity syndrome in infected chickens where adiposity was accompanied by relatively low levels of serum cholesterol and triglycerides compared to controls (Dhurandhar *et al*, 1992). Ten of 52 subjects were

positive for SMAM-1 or cross-reacting antibodies. As with SMAM-1-infected chickens, they featured obesity with hypolipidemia. The authors suggest that this study provides evidence that virus infections might be linked to obesity in humans (Dhurandhar *et al*, 1997).

The adipogenic potential of a human virus, the group D adenovirus AD-36, has been demonstrated in infected mice and chickens (Dhurandhar *et al*, 2000) where increased adiposity was accompanied by paradoxically low levels of serum cholesterol and triglycerides as in the SMAM-1 animal model. Routine histopathological examination of brain tissue from obese animals was stated to be unremarkable.

An obesity syndrome was found to develop in rats following infection with Borna disease virus (BDV) (Narayan *et al*, 1983). BDV, a negative-strand RNA virus of the family Bornaviridae, is known to cause central nervous system disease in a wide range of vertebrates including, possibly, humans (Hatalski *et al*, 1997; Nowatry and Kolodziejek, 2000). A neuropathological study (Gosztonyi and Ludwig, 1995) revealed only slight cellular inflammatory infiltration of obese brain specimens, except in the infundibulum of the hypothalamus, a structure outside the blood–brain barrier. As the syndrome developed, viral antigen-positive cells decreased progressively, as also observed in the CDV model. Progressive involution of the hippocampal formation, a hallmark of experimental BDV infection, occurred. Vacuolar degeneration of neurons in the hypothalamic paraventricular nucleus (PVN) was frequently found in obese brain specimens, in the absence of inflammatory changes.

Recently, the expression of various neuropeptides implicated in the regulation of energy homeostasis has been evaluated by immunohistochemistry in rats infected with an obesity-inducing BDV strain (Herden *et al*, 2000). Expression of α-MSH was found to be reduced in infected animals compared to uninfected controls. Hypocretin and neuropeptide Y (NPY), in contrast, were found at comparable levels in both infected and control groups. Both NPY and hypocretin both promote increased energy intake in contrast to the anorexogenic effect of α-MSH (Schwartz *et al*, 2000). As in the case of CDV-related obesity, it is possible that BDV selectively targeted arcuate-POMC neurons, source of the α-MSH peptide.

The induction of obesity in mice inoculated with the scrapie agent was shown to develop slowly over several weeks prior to the onset of clinical scrapie and was related to the strain of the agent rather than mouse strain. (Carp *et al*, 1998). The adrenal gland was the only organ showing a significant increase in weight (due to expansion of the cortex) and adrenalectomy was shown to prevent obesity development (Kim *et al*, 1988). It was suggested that scrapie-induced obesity depended on the effect of the agent on the hypothalamic-pituitary-adrenal axis. It is notable here that adrenalectomy ameliorates obesity-related phenotypes in most genetic and other experimental models of rodent obesity (Leibel *et al*, 1997)

Discussion and summary

In four of the five animal models of postinfectious obesity discussed, the evidence, although incomplete, appears to suggest that neuroendocrine dysregulation, centrally mediated, is playing a pivotal role in disease development. Differences in phenotype are notable. Thus, defective regulation of thyroid function in RAV-7 induced obesity in chickens was of major significance as was adrenal gland dysfunction and putative steroid dependence of the obese phenotype in scrapie-induced obesity. In the case of adenovirus-induced obesity, it is possible that additional neuropathological studies will provide evidence of a subtle hypothalamic lesion. A more coherent picture is beginning to emerge with respect to the pathogenesis of CDV- and BDV-induced obesity syndromes. This has been greatly facilitated by the identification over the past few years of new signalling molecules and their receptors, which have helped define, at least in part, the hypothalamic pathways regulating feeding and body weight.

CDV-induced obesity was found to be associated with a marked reduction in POMC mRNA transcription in arcuate-POMC neurons. The likely cause is believed to be either cell loss due to viral cytopathic effects or/and reduced signal transduction due to diminished leptin receptor expression in the cells. ARC-POMC neurons are now known to represent a critical population in establishing the arcuate nucleus as a major site for transducing afferent regulatory signals from the blood (such as leptin and insulin) into a neuronal response. POMC neurons are the source of the precursor protein from which the melanocortin peptides such as α-melanocortin-stimulating hormone (α-MSH) are derived by proteolytic cleavage. These are known to inhibit energy intake as noted earlier. The α-MSH receptor (MC4-R) is located in several hypothalamic nuclei but is present in very high density in the PVN, where it is believed to represent an important downstream target in the leptin signaling cascade (Sealey *et al*, 1997). As part of this central melanocortin system, agouti-related protein (AGRP), colocalized with neuropeptide Y in another set of arcuate neurons that project to the same PVN cells, serves as a competitive antagonist to the melanocortin agonist. Together they are thought to be involved in the regulation of energy homeostasis in mice and humans, a concept supported by studies of monogenic obesities as well as transgenic and knockout animal models. (Chen and Garg, 1999; Cowley *et al*, 1999).

In the CDV-model, levels of α-MSH and its receptor were not measured, although as noted previously expression of α-MSH was found to be reduced in

animals infected with an obesity-causing strain of BDV. The possible effect of virus-induced deletion of cell bodies in the dorsomedial and ventromedial hypothalamus on the suggested modulation of leptin signalling by arcuate POMC and NPY/AGRP cells (DeFalco *et al*, 2001) remains unknown. Although incomplete, the findings with the CDV and, likely, the BDV models suggest that obesity was related to a lesion of the central melanocortin feeding center in the hypothalamus.

The association of reduced numbers of dopaminergic cells and reduced dopamine levels in obese brain specimens, although not understood, is likely to be of significance. Remarkably, these cells, of the tuberoinfundibular DA system, which largely project to the median eminence and pituitary, appear to be similarly dysregulated in the ob/ob mouse (Oltmans, 1983). In the latter model (of monogenic obesity) it has recently been shown that dopaminergic agonists normalize body weight gain, hyperglycemia, and elevated hypothalamic NPY (Bina and Cincotta, 2000).

Reports of human obesity syndromes developing as late sequelae of acute viral infection are meager. Von Economo, in describing the late sequelae of encephalitis lethargica, presumably of viral origin, noted a 10% incidence of morbid obesity in survivors, while postmortem histological examination of the brains of some patients revealed only nonspecific changes (Von Economo, 1918; Hall, 1924; Hirsch, 1984). Noteworthy in our experiments was the absence of gross histological changes in the brain of many obese animals; had the fact of an initial virus infection not been known, there would be no virological or histological evidence that the observed obesity syndrome had its origin in a preceding viral infection.

The potential neuroinvasiveness of many of the common viruses encountered in childhood is well established. Of viruses specifically implicated in childhood encephalopathies are enteroviruses, such as certain ECHO and coxsackie virus serotypes, herpes viruses, respiratory viruses such as respiratory syncytial virus, and influenza A and measles virus (Kennedy *et al*, 1986). Whether any of these viruses have intrinsic adipogenic potential is not presently known. It seems unlikely that the Ad-36 virus will remain as the sole example of a human obesigenic virus.

Although evidence for direct involvement of hypothalamic systems in the previously mentioned childhood infections is lacking, hypothetically, by analogy with the postinfectious models, subtle hypothalamic damage affecting central energy regulating systems, remains a possibility.

Detailed examination of autopsied brain specimens from morbidly obese human subjects, focussing on the mediobasal hypothalamus, and the use of immunohistochemical staining for GFAP, TH, leptin receptors, as well as molecular screening for genome sequences of common viruses by polymerase chain reaction and *in situ* hybridization, may provide clues pointing to an infectious etiology for some cases of morbid obesity in humans.

Further exploration of "slow-virus" models of obesity—where the environmental influence is a commonplace infectious agent acting in a host lacking any identifiable genetic abnormality or predisposition—is expected to provide additional insights into pathogenetically relevant neuronal cell groups, networks, and signalling molecules and their receptors.

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