Novel Interventions for the Control of Disease-Related Anorexia

Wolfgang Langhans

Physiology and Animal Husbandry, Institute of Animal Sciences, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland

Anorexia is a common feature of many diseases. In the initial phase of systemic infections, the anorexia is part of the generalized host defense reaction, which is termed “acute phase response” (APR) (1). The APR is characterized by alterations in immune, endocrine, metabolic, and neural functions, and it limits the proliferation and spread of the infectious agent. Initially, the accompanying anorexia is also beneficial for the host in various ways (2,3). This is reflected by the decreased survival time and increased mortality that force-feeding causes in experimentally infected mice (4). The anorexia in the acute phase of an infectious disease should therefore not be treated unless a patient is in poor condition. Chronic anorexia, however, which is observed during cancer and other chronic diseases, compromises host defenses and is ultimately deleterious. In such cases therapeutic measures are indicated. Based on current knowledge about the general mechanisms of disease-related anorexia, I will discuss some therapeutic options to counteract it.

MECHANISMS OF DISEASE-RELATED ANOREXIA

General Aspects

The mechanisms underlying disease-related anorexia have been investigated using several models of inflammation, infection, or cancer. Sterile inflammation is often produced in laboratory animals by subcutaneous injection of turpentine, which results in fever, lethargy, body weight loss, muscle breakdown, and anorexia (5). Intrarectal administration of irritants such as trinitrobenzenesulfonic acid (TNB) is used to model inflammatory bowel diseases, such as ulcerative colitis and Crohn’s disease. These diseases are usually associated with marked weight loss due to reduced food intake, malabsorption, and increased energy expenditure. Similarly, TNB-treated animals typically eat less and lose body weight (6). Peripheral administration of live bacteria or bacterial lipopolysaccharide (LPS) is widely used to model systemic bacterial infections (7-9). LPS are Gram-negative bacterial cell wall constituents that are released after bacteriolysis or during periods of rapid proliferation (10); they
Disease-related anorexia can be caused by various factors including tumor, inflammation, and infection. Key pathways and molecules involved in anorexia include:

- **Pro-inflammatory cytokines** (e.g., IL-6, IL-12, IL-18, TNFα)
- **Calcium antagonists**
- **Glucocorticoids**
- **ODN**
- **PDE inhibitors**
- **PPAR ligands**
- **sCD14**
- **sCR**

Molecules and pathways that can modulate anorexia include:

- **IFNγ**
- **IL-12, IL-18**
- **COX-2 inhibitors**
- **Prostanoids**
- **GLP-1 antagonists**
- **CRF, αMSH**
- **5-HT antagonists**
- **5-HT**

The brain capillary endothelial cells are also involved, and the hypothalamus plays a role in this process. The diagram illustrates the complex interplay of these factors and pathways in the development of anorexia.
trigger most of the host's reactions to severe Gram-negative bacterial infections and generalized inflammation. Inoculation of mice with *Toxoplasma gondii* cysts has emerged as another infection model that allows the differentiation between the ano-rectic and hypermetabolic phases of cachexia (11). Finally, several experimental tumors are available to model cancer cachexia and anorexia (12). All these models have in common that they are usually associated with acute or prolonged anorexia, hypermetabolism, and at least a transient increase in circulating concentrations of pro-inflammatory cytokines. In addition, weight loss is observed with the chronic models of anorexia.

**Role of Cytokines**

Pro-inflammatory cytokines are involved in both protection against and pathogenesis of the disease. They inhibit eating after peripheral and central administration (13,14) and are considered to be the major endogenous mediators of disease-related anorexia. A synergistic feeding suppressive effect has been described for interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) (15,16), and for these two cytokines and IL-8 (17). This synergism is presumably related to the cytokines' overlapping effects and to the fact that they act through converging intracellular signaling pathways.

Results from experiments in which the synthesis of particular cytokines or their receptors was acutely antagonized by receptor antagonists, soluble receptors, or pharmacologically active substances implicate many pro-inflammatory cytokines in the anorexia during disease (18–21). Furthermore, experiments in mice genetically deficient of a particular cytokine or cytokine receptor established a crucial role for several cytokines in the anorexia accompanying some models of disease. IL-6 appears to be essential for the anorexia in the SC turpentine model of sterile local inflammation (22,23), and interferon-γ (IFN-γ) is a major contributor to the anorexia in response to peripheral LPS administration (18). Cytokine or cytokine receptor knockout (KO) mice also display differences in the magnitude of anorexia and cachexia induced by some experimental tumors (12,24). The also observed failures to establish a role of particular cytokines in disease-related anorexia with KO mice (13,25–27) are presumably due to the redundancy and overlapping actions of cytokines that allow for developmental compensation. All in all, it is safe to say that pro-inflammatory cytokines play a prominent role in the anorexia of various diseases.

**FIG. 1.** Diagram of some major pathways of disease-related anorexia, incorporating pharmacologic intervention options. See text for further details. 5-HT, serotonin; α-MSH, alpha-melano-cyte stimulating hormone; cAMP, cyclic adenosine monophosphate; COX-2, cyclooxygenase-2; CR, cytokine receptors; CRF, corticotropin-releasing factor; GLP-1, glucagon-like peptide-1; IL-1 [or 6,12,18], interleukin-1 [or 6, 12, 18]; IFN-γ, interferon-γ; IL-6, IL-12, IL-18; NF-κB, nuclear factor-κB; ODN, oligo-deoxynucleo-tides; PDE, phosphodiesterase; PPAR, proliferator activated receptor; SOCS-1, suppressor of cytokine signaling-1; TLRs, toll-like receptors; TNF-α, tumor necrosis factor-α; O, inhibition.
Sites of Cytokine Action

Peripheral immune stimulation and/or cytokines activate central nervous system (CNS) regions involved in the control of eating (28,29). Some pro-inflammatory cytokines and their accessory proteins are expressed by neurons and glia cells in normal brain (30), and CNS cytokine production is promoted during acute and chronic CNS diseases (e.g., meningitis, encephalitis, multiple sclerosis, Alzheimer's disease, stroke, and brain tumors) (31,32). Intracerebroventricular (ICV) administration of pro-inflammatory cytokines (17) presumably models the clinical features and anorexia of such diseases.

Pro-inflammatory cytokines synthesized in the brain also appear to be involved in some centrally controlled phenomena in response to peripheral immune stimulation (33-37). This includes the anorexia in certain models of cancer (38,39) and in response to acute colitis induced by rectal administration of TNB in the rat (40). Often, however, a crucial role of centrally produced cytokines in the anorexia accompanying systemic diseases is questionable because an essential cytokine is not expressed in the brain (18) or because the expression pattern of the cytokines and cytokine receptors is inconsistent with a role in anorexia (35), because "supraphysiologic" peripheral stimuli have to be used to reliably induce CNS cytokine synthesis (37,41,42), or because the time course or localization of CNS cytokine expression does not fit a role in anorexia (41,43,44). Several results from genetic (45,46) or pharmacologic (19,47) antagonism studies also argue against a role of central cytokines in the anorectic response to peripheral diseases. Thus, some peripheral diseases may stimulate cytokine synthesis in the brain, but in general, they do so mainly outside the blood-brain barrier (BBB). Those peripheral cytokines can reach CNS cytokine receptors through active or passive transport mechanisms (48) or through circumventricular organs (49). The latter possibility appears to be of minor importance for the mediation of disease-related anorexia (50-52). Also, neural afferent pathways do not appear to be essential for the feeding suppressive effects of circulating cytokines (53). Circulating cytokines may, however, influence food intake by acting on brain capillary endothelial cells (54). Those cells and perivascular cells (e.g., microglia, macrophages) possess cytokine receptors (55-57). The binding of pro-inflammatory cytokines to these receptors leads to activation of the transcription factor nuclear factor-κB (NF-κB) and to the subsequent release of messengers, such as nitric oxide or prostanoids (55,57-61), which modulate neural activity.

OPTIONS FOR INTERVENTION

General Aspects

Cytokines are natural targets for therapeutic intervention because of their key function in the pathogenesis of many diseases. Given the cytokines' multiple actions and target sites, it is not surprising that their activities are tightly controlled by physiologic feedback loops. These natural control mechanisms can theoretically be
exploited to modulate cytokine actions, and several specific options for pharmacologic intervention are feasible. Cytokine activity can be controlled at the levels of i) cytokine production (i.e. pre- and posttranscriptionally), ii) cytokine receptor expression, iii) the cytokines' ability to interact with their receptors, and iv) at the downstream signaling pathways. Given the complexity of the cytokine network, difficulties can be expected in determining the right time of application and the appropriate dose of a potential antagonist. The theoretical dissociation in upstream (cytokine production) and downstream (cytokine action) controls of cytokine action is not always clear because the pathways of cytokine production and cytokine action often converge on the same transcription factors. Accordingly, several inhibitors of cytokine effects may act upstream and downstream of cytokines through a modulation of those transcription factors. Therefore, I will categorize the intervention options discussed below in 1) control of membrane binding, 2) control of intracellular signaling, and 3) control of neurochemical pathways.

Control of Membrane Binding

**Toll-Like Receptors/CD14**

One well-known pathway for the stimulation of pro-inflammatory cytokine synthesis by disease processes is their induction by bacterial products such as LPS. LPS has no structural homologue in mammalian organisms and is a potent stimulus of innate immune reactions. The activation of macrophages by LPS requires the cell surface glycoprotein CD14 and is promoted by the LPS-binding protein (LBP) (62). In cells devoid of CD14, such as endothelial cells, the soluble form of CD14 (sCD14) present in serum can replace membrane-bound CD14 (63). We recently found that CD14 KO mice are completely insensitive to the feeding suppressive effect of intraperitoneally (IP) injected LPS (Von Meyenburg et al. unpublished), illustrating that CD14 is also essential for LPS-induced anorexia. The actual LPS receptor, however, is the Toll-like receptor-4 (TLR-4) (63,64), whereas the TLR-2 has been implicated in the responses triggered by Gram-positive bacteria (65). TLRs are a family of transmembrane proteins that are grouped into the same gene family (63,64). The postreceptor signaling pathways of TLRs involve the adapter protein MyD88 and the Ser/Thr kinase IRAK, which interacts with TRAF6 and leads to the activation of the transcription factors NFκB and activating protein-1 (AP-1) (66). The transcription factors stimulate the transcription of the genes for prostanoids, pro-inflammatory cytokines, and other downstream mediators of LPS effects. Currently (early 2002) the signaling pathways from CD14/TLR4 to NFκB have not been specifically explored with respect to their potential for therapy, but this may soon happen. In principle, it should be possible to design drugs that target specific TLRs, CD14, or the downstream signaling mediators and are thus able to mitigate the worst consequences of microbial infections. Interestingly, some evidence indicates that LBP and sCD14 not only mediate LPS effects, but that administration of exogenous LBP
and sCD14 can also mitigate excessive responses to LPS (62,67) and might therefore hold some therapeutic potential.

**Cytokine Receptors**

Many cytokine receptors (CR) are naturally expressed as both membrane and soluble forms. Soluble cytokine receptors (sCR) are created by proteolytic cleavage of membrane receptors (receptor shedding) or alternative splicing of the RNA encoding the CR, creating a truncated, soluble receptor (68). As the affinities of the sCR usually match those of the membrane receptors, they are potent binding competitors. Most sCR therefore act as cytokine antagonists and have generated interest as potential immunotherapeutic agents. Advantages of sCR as therapeutic agents are their high specificity for the target cytokines, their low immunogenicity, and their few side effects compared with other immunosuppressive agents such as glucocorticoids (68). The use of sCR as therapeutic agents is limited, however, by their relatively short biologic half lives; by their carrier effects for cytokines, which may cause undesired side effects on dissociation of the sCR-cytokine complex; by their high molecular weight protein nature, which makes them susceptible to proteolytic degradation and presumably prevents crossing of the BBB; and by their high costs (68). In sum, cytokines with a dominant role in disease-related anorexia may offer the best chances for successful therapeutic intervention with sCR. The future must show whether sCR can become practically relevant pharmaceutical tools, which might eventually also be used to counteract disease-related anorexia.

**Control of Intracellular Signaling**

**Cyclic Adenosine Monophosphate**

It is well known that intracellular cAMP counteracts cytokine-mediated inflammatory responses. One way to increase intracellular cAMP is to inhibit the enzyme phosphodiesterase (PDE) IV, which degrades cAMP. Nonspecific PDE inhibitors, such as pentoxifylline (PTX), have been in clinical use for many years and are known to inhibit TNFα production in response to several pathologic states and models of disease (69–73). We found that PTX blocked the anorexia in response to IP injection of LPS in rats (20). This was accompanied by a complete blockade of the production of TNF-α and by an attenuation of IL-1β production (20). Under the same conditions, PTX did not influence the feeding suppressive effect of exogenous TNF-α. These results indicate that TNF-α plays a substantial role in peripheral LPS-induced anorexia and that, at least in this model of LPS anorexia, PTX does not act downstream of TNF-α production.

Depending on the cellular environment and the disease processes that trigger cytokine production, PDE inhibitors may upregulate, downregulate, or have no effect on production of IL-1 and other cytokines in infection and inflammation models (72,74). PTX has also been shown to inhibit LPS-induced production of IL-18 in
mice and the synergistic induction of IFN-γ by combined IL-12/IL-18 treatment in vitro and in vivo (75). These effects might contribute to the elimination of LPS-induced anorexia by PTX because IFN-γ has been identified as a major endogenous mediator of LPS anorexia (18).

The molecular mechanisms by which PTX and other compounds affect cytokine synthesis are not completely resolved. PTX increases intracellular cAMP by preventing its degradation, which suppresses TNF-α production (76,77). An increase in endogenous adenosine may also contribute to the inhibition of TNF synthesis by phosphodiesterase inhibitors (73). Siegmund et al. (78) recently demonstrated that systemic administration of an adenosine kinase inhibitor markedly improved all clinical scores in a mouse model of colitis. These improvements appeared to be related to an inhibition of IFN-γ synthesis (78). Food intake was not measured in this study, but it appears reasonable to assume that it was also improved. There is good reason to believe that activation of the cAMP/PKA cascade and, hence, the mitigating effects of PDE inhibitors on pro-inflammatory cytokine actions are ultimately related to the interference of these substances with the transcription factor (NFκB) (79).

PTX decreased the loss of body weight and muscle protein observed in infected animals (69). Recently, it was also shown that PTX prevents muscle atrophy and suppresses increased protein breakdown in tumor-bearing rats by inhibiting the ATP-dependent ubiquitin proteasome pathway of proteolysis (80). Moreover, PTX appears to attenuate the inhibition of insulin effects by pro-inflammatory cytokines (81). Finally, PTX has been shown to not decrease hepatic acute phase protein synthesis and to increase production of the anti-inflammatory cytokine IL-10 (72).

All in all, PTX is an established drug with no severe side effects (80) and may improve therapeutic strategies in a variety of clinical situations, such as sepsis (82), rheumatoid arthritis (83), acquired immunodeficiency syndrome (84), and even cancer, to some extent (71). With respect to the potential therapeutic use against disease-related anorexia, the comparatively specific effect of PTX on the synthesis of particular cytokines is very positive because it should not compromise other defense reactions. All in all, PDE inhibitors, alone or in combination with other agents, should provide a viable option to treat disease-related anorexia.

**Calcium (Ca²⁺)**

Various calcium (Ca²⁺) antagonists (Ca²⁺ channel blockers and dantrolene) have protective effects in cytokine-mediated pathophysiologic processes. They improve survival in endotoxemic animals and improve metabolic abnormalities associated with sepsis (85). Many of the beneficial effects of Ca²⁺ antagonists appear to be related to an inhibition of cytokine production. Ca²⁺ antagonists decrease the production of the pro-inflammatory cytokines TNF-α and IL-1 (86,87) and increase the production of the anti-inflammatory cytokine IL-10 (87). Interestingly, dantrolene, but not verapamil or diltiazem, suppressed LPS-induced production of IL-12 and
IFN-γ (86), suggesting that Ca\(^{2+}\) inflow from the extracellular space or from intracellular storage sites affects cytokine production differently. In our hands, pretreatment with the Ca\(^{2+}\) channel blocker verapamil inhibited the anorectic effect of IP LPS (88), suggesting that a Ca\(^{2+}\)-sensitive mechanism related to the influx of extracellular Ca\(^{2+}\) is involved. In contrast, verapamil did not block the feeding suppressive effect of exogenous TNF-α (89), which is consistent with the assumption that verapamil antagonizes LPS anorexia by inhibiting TNF-α production. Some evidence suggests that Ca\(^{2+}\) antagonists also modulate the inflammatory response by reducing the production of superoxide (SO) and nitric oxide (NO) by macrophages (90). Finally, dantrolene attenuated the muscle breakdown associated with abdominal sepsis in rats (91), and reduced serum TNFα and corticosterone, muscle Ca\(^{2+}\), and mRNA levels for the muscle specific calpain p94, as well as total and myofibrillar protein breakdown rates (91). These results suggest that decreased levels of TNF-α and glucocorticoids contribute to the anti-catabolic effects of dantrolene during sepsis. Thus, Ca\(^{2+}\) antagonists provide another promising therapeutic option for the anorexia in disease. Their potential in this context deserves to be further explored.

Transcription Factors/Nuclear Factor κ-B

Bacteria, viruses, bacterial products, and cytokines lead to a marked activation of the transcription factor NF-κB (92). NF-κB is induced by many different stimuli and it participates in the regulation of more than 150 target genes. Because NF-κB regulates the expression of pro-inflammatory cytokines, chemokines, and other immune factors, NF-κB has often been termed a "central mediator of the immune response" (92). The selectivity of NF-κB activation resides mainly in the cell type that is targeted by the activating stimulus. In relation to disease-related anorexia it is therefore interesting that bacterial products as well as pro-inflammatory cytokines and the sterile inflammation model of intramuscular turpentine administration induce a strong activation of NF-κB in brain capillary endothelial cells (93). Numerous inhibitors of NF-κB activity have been described. For many of those molecules, the exact mode of action is not clear yet. Some inhibitors affect NF-κB-induced DNA binding and a variety of downstream pathways. This overlap makes it a challenge to find molecules that block specific pathways without interfering with other signaling cascades (94). One future goal in relation to disease-related anorexia and other CNS-mediated phenomena should therefore be to design molecules that inhibit distinct NF-κB complexes in brain capillary endothelial cells.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily. PPAR-α usually controls intra- and extracellular lipid metabolism, whereas PPAR-γ triggers adipocyte differentiation and promotes lipid storage. Recent findings indicate that both PPARs also modulate the inflammatory response (95). PPAR-α ligands inhibit IL-1β-induced IL-6 secretion and prostaglandin production in aortic smooth muscle cells (96), and PPAR-γ ligands were shown to inhibit TNF-α, IL-1β, and IL-6 expression in monocytes (97). In general, PPAR activators have been shown to exert
anti-inflammatory activities in various cell types by inhibiting the expression of pro-inflammatory genes. They do so by negatively regulating the transcription factors NF-κB and AP-1 and by stimulating the catabolism of pro-inflammatory eicosanoids (95). Yet, there are also conflicting reports concerning the anti-inflammatory actions of PPAR ligands (98), and some evidence indicates that PPAR activators may even cause undesired side effects (99). Thus, although PPAR activators may have some therapeutic potential to treat cytokine-mediated disease phenomena (100), further studies are necessary to critically examine the situational variability of these effects and to test whether PPAR ligands provide a realistic option for the treatment of disease-related phenomena such as anorexia.

A recently developed strategy for the modulation of endogenous transcription is the “decoy strategy” in which transfection of a double-stranded oligo-deoxynucleotide (ODN) corresponding to the cis sequence results in attenuation of the authentic cis-trans interaction with subsequent modulation of gene expression (101). Using this technique, Kawamura et al. (101) recently showed that intratumoral injection of ODN to the NF-κB binding site partially prevented the anorexia and eliminated the cachexia in a mouse tumor model. Meanwhile, the same strategy also worked in another tumor model with metastases (102). These interesting findings may eventually open new therapeutic options to counteract disease-related anorexia and cachexia.

A more conventional way to inhibit NF-κB–induced transcription of pro-inflammatory genes is by administration of glucocorticoids. Glucocorticoids inhibit bacterial product-induced anorexia (103). Recent advances in our knowledge of how glucocorticoids act may provide new opportunities for the development of new treatments (104). Dexamethasone inhibited the synthesis of pro-inflammatory cytokines induced by peripheral LPS in brain capillary endothelial cells (105). Therefore, glucocorticoid treatment for the anorexia of acute bacterial infections might be beneficial in some cases. However, there still remains the question of the specificity of glucocorticoid actions, and many of the side effects of glucocorticoids preclude their sustained administration (104).

Suppressors of Cytokine Signalling

The fact that IFN-γ plays a crucial role in some cases of disease-related anorexia is interesting with respect to a new group of endogenous cytokine antagonists termed suppressors of cytokine signaling or SOCS (106,107). SOCS act as negative regulators of the JAK/STAT cascade, which is a key cytokine-activated signaling pathway (106). IFN-γ specifically upregulates the expression of SOCS1, an important negative regulator of IFN-γ, thereby limiting its own activity (107). This built-in limitation of IFN-γ activity is essential for normal development and survival because SOCS1 KO mice die before 3 weeks of age (108). Recently, it has been shown that SOCS1 is required for the timely attenuation of IFN-γ activity (109). In sum, SOCS are intracellular molecules that represent a classical negative feedback loop by terminating the responses to the potentially damaging effects of cytokines. It will be
interesting to see whether they can be further exploited as therapeutic agents against disease-related phenomena such as anorexia.

**Cyclooxygenase-2**

Nonspecific inhibition of cyclooxygenase (COX) has been shown to attenuate the feeding suppressive effects of exogenous IL-1β and LPS (88,110,111) as well as cancer anorexia and cachexia (12). Indomethacin also blocked the activation of hypothalamic neurons by LPS and IL-1β (52,112). These data suggest that eicosanoids produced around the BBB are involved in the anorexia during various diseases. Cytokines and LPS increase the expression of COX-2 mRNA, the inducible form of COX, in endothelial cells of brain capillaries (59,113–116) and potently stimulate the production of eicosanoids—in particular PGE₂—by these cells.

We could recently show that the anorectic effect of IP LPS is blocked by the COX-2 inhibitor NS-398, but unaffected by the COX-1 inhibitor resveratrol. Under the same conditions, peripheral LPS markedly increased CSF PGE₂ concentration, but not plasma PGE₂ (117), and this effect was also blocked by NS-398, but not by resveratrol. These data are consistent with the idea that CSF PGE₂ is somehow involved in the feeding suppressive effect of peripheral LPS. Inhibition of COX-2 may therefore be a promising option to selectively counteract the centrally mediated effects of pro-inflammatory cytokines, such as the anorexia, while leaving the peripheral and, hence, many of the beneficial defense functions of cytokines untouched. COX-2 inhibition has been shown to reduce tumour growth and cachexia in some models of cancer (118,119). Finally, the role of COX-2 in disease-related anorexia may also open the possibility of nutritional interventions with ω-3 polyunsaturated fatty acids, which influence prostanoid production. This approach, however, may be difficult because of the variability of the effects of polyunsaturated fatty acids on cytokine production.

**CONTROL OF NEUROCHEMICAL PATHWAYS OF ANOREXIA**

**General Aspects**

Antagonism of the downstream effector neurons of pro-inflammatory cytokines is a particularly attractive option to mitigate disease-related anorexia because it does not carry the risk of interfering with the defense functions of cytokines. PGEP₃ receptors, which mediate the neuromodulatory effects of PGE₂, have been located on medullary but not on paraventricular (PVN) neurons (52). IP LPS, and IL-1β stimulated the ex vivo release of PGE₂ from brainstem slices (120), and microinjection of PGE₂ into the rostral ventrolateral medulla provoked cellular activation in the PVN (52). All these data suggest that prostanoids mediate the effect of circulating cytokines on hindbrain neurons, which project to the hypothalamus.

**Serotonin**

Serotonin (5-HT) is abundant in neurons originating from the midbrain dorsal raphe nucleus and the hindbrain, projecting to the hypothalamus, in particular to the
PVN and ventromedial nucleus (VMH). 5-HT is thought to inhibit eating through the 5-HT1b and/or 5-HT2c receptors (121). PG EP3 receptors have been located on serotonergic neurons in the raphe nucleus and hindbrain (122). Those neurons project to the hypothalamus and are activated by PGE2 (122), which makes them top candidates for the mediation of bacterial product and circulating cytokine-induced anorexia. The dorsal raphe nucleus also contains IL-1 receptors type 1 (123), and central as well as peripheral administration of IL-1B and TNF-α increases serotonergic activity in this area (124). Peripheral IL-1B stimulates 5-HT turnover also in the hypothalamus (125). 5-HT appears to mediate the feeding suppressive effect of rectal TNB administration in the rat (126). In our hands, 8-OH-DPAT and ritanserin, compounds that reduce 5-HT synthesis or transmission, attenuated the anorectic effect of LPS and IL-1β after peripheral and central administration (127). Administration of 8-OH-DPAT directly into the dorsal raphe nucleus also blocked the feeding suppressive effect of peripheral IL-1β (117). All these data are consistent with the hypothesis that serotonergic neurons originating in the hindbrain play a role in mediation of the cytokine-induced inhibition of feeding. On the other hand, Swiergiel and Dunn recently reported that numerous 5-HT antagonists did not attenuate IL-1β-induced suppression of short-term milk intake in mice (128). Whether this discrepancy is due to a species difference or to method differences is not clear. CNS 5-HT antagonism also yielded conflicting results concerning cancer anorexia (attenuation of anorexia: [129], no change in anorexia: [130]). In general, the mediation of cytokine-induced suppression of feeding by central serotonergic systems appears to be situationally variable and may be related to changes in serotonergic systems in several brain areas. Clearly, however, there is some therapeutic potential of 5-HT antagonists that deserves to be further explored.

Glucagon-Like Peptide-1

In addition to ascending aminergic pathways, peptidergic neurons, which link the hindbrain and the hypothalamic PVN, appear to mediate signals related to eating. Rinaman and colleagues have shown that LPS activates GLP-1 neurons (131), and that GLP-1 neurons project to the PVN (131). More recently, the same group also showed that ICV administration of a GLP-1 receptor antagonist enhanced the fever inducing and attenuated the feeding suppressive effects of IP LPS (132,133), suggesting that GLP-1 is in fact involved in mediation of these LPS-induced phenomena. Whether the same holds true for cytokine-induced changes and can be exploited for successful therapy awaits further clarification.

Hypothalamic Neurochemical Pathways

Peripheral injection of IL-1β increases hypothalamic CRF mRNA (134) and IL-1β–induced anorexia was attenuated by ICV administration of a CRF antagonist (135), suggesting that CRF is involved in the neurochemical mediation of IL-1β–induced anorexia. Interestingly, prostanoids appear to mediate the effect of IL-1β
on hypothalamic CRF release (136), which might provide a link between the presumed roles of PGE2 and CRF in the feeding suppressive effect of IL-1β.

An antagonistic interaction between IL-1β and NPY has also been suggested, and that the feeding suppressive effects of IL-1β and tumors are in part due to a modulation of NPYergic mechanisms. Thus, it is possible that a cytokine-induced decrease in NPY attenuates feeding that would normally occur in response to an energy deficit (137).

Finally, LPS stimulates the release of αMSH (138). Alpha-MSH antagonizes inflammatory and acute phase reactions at various levels (cytokine production, cytokine action) in the periphery and the brain (139). Alpha-MSH binds to central melanocortin receptors (MC3-R and MC4-R). Central administration of MC4-R agonists inhibits food intake, increases energy expenditure, and reduces body weight (140). In contrast, deletion of the MC4-R increases food intake and body weight (141). Huang et al. (142) reported that αMSH enhanced LPS-induced anorexia in rats, whereas administration of the melanocortin (MC3/MC4) receptor antagonist SHU9119 attenuated it. More recently, the anorexia and cachexia induced by IP LPS or by a syngenic carcinoma were attenuated in MC4-R KO mice and also by the MC3-R/MC4-R antagonist agouti-related peptide (143). These data implicate the central melanocortin system in mediation of cytokine-induced anorexia and suggest some interesting therapeutic options based on powerful MC receptor antagonists.

INTEGRATION

The identification of the extracellular and intracellular mechanisms mediating disease-related anorexia and their downstream neurochemical pathways have suggested several options for successful pharmacologic intervention. Whereas some inhibitors of cytokine production and/or action are clinically well-established drugs with few side effects, other promising molecules are still at the experimental stage. It will be interesting to see which of those newly discovered substances or intervention principles will find their way from the bench to the bedside in the near future. One problem of anti-cytokine therapy for disease-related anorexia is that it often carries the risk of also antagonizing the beneficial defense functions of the cytokines. This risk does not apply to interventions aimed at blocking the neurochemical pathways of disease-related anorexia, which may therefore often be a less problematic means of successful pharmacologic intervention.

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**DISCUSSION**

*Dr. George A. Bray:* Thank you for enlightening me in lots of parts of the control of anorexia. I will talk in a few minutes about the opposite side of the coin and the question that arises is whether anybody has attempted, in a controlled fashion, to use these mechanisms for treatment of obesity. You were talking about trying to prevent this as a consequence of chronic malnutrition states, but the flip side would be to use these strategies for treatment of people who aren't anorectic, but who in fact are hyperphagic. Has that been tested, that you know of, anywhere? I haven't seen it yet.

*Dr. Wolfgang Langhans:* Well, to the best of my knowledge, this has never been attempted. Of course the modulation of the neurochemical mediators has been attempted, and we all know this is part of the ongoing research in control of food intake and energy balance, but if you go further upstream to the immunomodulators or immunologically active substances, then of course you have all kinds of other effects that you probably don't want in relation to obesity treatment. So I think it's understandable that it has not been tried.

*Dr. Antonius Holly Pudjiadi:* I think you are using a very simplistic model because firstly, cytokine signaling is perhaps necessary for body protection. TNF is only one of the enormous number of proteins secreted by many different cells to mediate signaling between cells. Secondly TNF-α usually only increased for several hours after mononuclear cells stimulation. The physiologic effects happen at end organ level. Many studies showed that blocking the TNF (for example with TNF receptor antagonist), without knowing what is going on, does
not give any significant clinical goal and may even make the condition worse. I suggest that we should block at a more terminal level of the pathway.

**Dr. Wolfgang Langhans:** Thank you very much. I agree with you. Blocking TNF is certainly not doing the trick, but this was pointed out by Anne already on Monday and I hope I also made the point that it's not a single cytokine. It doesn't help to block TNF-α with TNF receptor antagonists or something like that. It's important to consider here that this is a cytokine network. It doesn't help and it can even have negative effects to try to take out one of these cytokines, but many of the substances that I mentioned here don't just block TNF. So for instance I showed you some data with pentoxifylline. I know that the literature says, and we have also measured TNF in response to LPS and pentoxifylline, that you can block TNF release completely, but you also interfere with the release of other cytokines. For instance, when we use pentoxifylline we reduce also IL-1β levels. As far as your first remark is concerned that the model is simplistic, well, I am afraid this is probably one of the short comings of all models, that they may be a little simplistic, but if you want to understand a mechanism and if, based on this understanding, you want to proceed to generating some promising treatment strategies, then you probably have to use a model. Otherwise I'm afraid you won't get anywhere. And I guess that the combination of these models, as they were shown by Anne on Monday, as I've showed today and as I'm sure other people could show you in relation to tumor cachexia and so on, the combination of these models and what we learn from them, this I think should be the strategy that will ultimately get us somewhere in terms of the treatment. And also in relation to the cytokine issue, I think it's safe to say that cytokines in a combination, or the interaction of these cytokines, play a role in all these models, which doesn't mean that it's always the same mechanism. You could compare that to the role of gastrointestinal peptides in the control of food intake. Depending on the composition of the diet that you eat, you will activate a different "cocktail" of gastrointestinal peptides and you will ultimately get satiety as a result. It's a little bit similar here: Depending on the disease model or on the clinical disease at hand, a different "cocktail" of cytokines will be activated, and these cytokines then ultimately give you similar responses again. So, it's indeed much more complicated, and I fully agree with you that these models are somehow simplistic, but it's a starting point.

**Dr. Gareth Williams:** I've got one comment and a question. The comment is about your Fos positive cells that lit up in PVN. They're probably CRF neurons. You probably remember the work some years ago from Nancy Rothwell's lab, where they found that the thermogenic and pyrexic effects of IL-1β were blocked by α-helical CRF 941, a CRF antagonist. Hence, they thought this was probably CRF-mediated, and the activated neurones lie exactly where the CRF cell bodies are.

**Dr. Wolfgang Langhans:** This could very well be, Gareth. On the other hand, I left out a few data from the Elmquist lab and from others showing basically that you can block the thermogenic effects of LPS and cytokines by doing a subdiaphragmatic vagotomy, but you cannot block the c-fos activation or the feeding suppression with a vagotomy, and you can also not block it, as far as I remember, by doing AP/NST lesions. Actually, we have done AP/NST lesions and we didn't see any effect on this LPS and IL-1 model of anorexia. Also, you can activate these neurons by microinjections of prostaglandin E into the hindbrain and you can block this activation by indomethacin, given into the hindbrain. So I think the effect is somehow related to these ascending neurons, but still, of course you are right, this could be CRF neurons that are activated in the hypothalamus. CRF of course could also be one of the downstream mediators of the feeding suppression and you may remember that it was on my list of potential candidates for the hypothalamic neurochemistry.
**Dr. Gareth Williams**: Could I ask a brief, philosophical question? You're talking about short-term conditions, which either get better or they don't. Is there actually any evidence that influencing appetite, restoring appetite during that relatively short febrile illness actually influences outcome? For example, if you give 8-OH-DPAT into the raphe nucleus so that you prevent the animals from undereating, do they survive the effects of the pyrogenic defects of the infection better?

**Dr. Wolfgang Langhans**: It's hard to answer that question because of the low LPS doses that we use; we didn't go to high doses where the animals don't survive, we just use this dose range which is considered to be "pathophysiologically relevant" because it doesn't approach a condition of endotoxin shock. So these animals never die.

**Dr. George Fuchs**: This issue of tolerance to LPS has always made me a little bit unsure of how to interpret some of these conceptual frameworks, as well as some of the results of some of these different models, because I don't see that any of the models, at least that I'm familiar with, really replicate what I think probably is going on in the clinical situation in which LPS is perhaps pulsed in terms of exposure to the host or intermittent host, and I just wondered if you could tell us what's the latest on this issue of tolerance. What's the time course, once the LPS has been removed, for the cells to regain responsiveness and how do you think this issue of tolerance might relate to our interpretation of the results in these models and in the clinical situation? Is it relevant or not relevant?

**Dr. Wolfgang Langhans**: You're perfectly right that this is a critical issue and actually there are some complicated results around, which are not easy to explain. I mentioned the tolerance phenomenon during my presentation. When you give repetitive LPS injections, you get tolerance. Animals don't reduce food intake anymore. However, we did another study, which may be clinically very relevant because you just said that you may have a pulsatile release or exposure to LPS or cytokines. So, we found when you have a pulsatory exposure to cytokines, you may have an enhanced effect, and we also saw this in the continuous infusion model of LPS, which may better mimic a clinical situation in which you have a kind of protracted exposure to LPS. When we pulse the animals with LPS afterwards, we don't have a desensitization. We have an LPS hypersensitivity instead, that means the animals react more when we give an LPS injection after continuous infusion. And in this case, I think this relates to your question concerning a possible clinical relevance. The latest development on the tolerance phenomenon in general is that this is somehow related, as far as I know at least, to changes in the number and/or sensitivity of the TLR-4.

**Dr. Anne Ballinger**: This follows on Gareth's question really. There is a suggestion that force-feeding of animals with an infection will increase their mortality. So although the dose of LPS doesn't cause mortality in your animals, did you look at whether this more natural increase in feeding had any effect on apoptosis or any of these things, which were considered to be detrimental to increased feeding?

**Dr. Wolfgang Langhans**: No, we did not, but it's not just a philosophical issue, it's a very interesting and relevant question. We should do that at some point.

**Dr. Aspi Irani**: I have a couple of clinical questions. Firstly, compensatory over-eating after recovery from an acute infectious or non-infectious disease is a common observation, especially with viral hepatitis as an example of an infectious disease, or diabetic acidosis as an example as a non-infectious disease. On recovery from these diseases, very soon after recovery, the children begin to over-eat and this appears to be a compensatory mechanism. Now what is the basis or mechanism of this? That is the first question. A corollary to this, traditionally improvement in appetite is considered a sign of recovery from a disease. So when we talk of treatment for anorexia during the disease, are we talking only of symptomatic
relief? And is not treatment of the primary condition more important? Another corollary to this corollary, during an illness, the body switches from the absorptive phase of metabolism to the starvation phase? Now feeding a child during the starvation phase of metabolism, would it serve any purpose, would the food be utilized?

**Dr. Wolfgang Langhans:** Let me try to answer your questions step by step. The first one was the compensatory over-eating. In the LPS model, a single LPS injection doesn’t change body weight and you don’t get weight loss, because the animals eat less only for about 24 hours, and this is a difference of about 10 g which doesn’t markedly affect body weight. So you don’t get compensatory over-eating in this situation. In the chronic LPS infusion model, which I presented earlier in the talk, we administered LPS through osmotic mini pumps, and there you get a marked decrease in body weight and some compensation afterwards. So, my conclusion would be that any compensatory overeating, after recovery from a disease, is somehow related to the mechanisms that were beautifully shown yesterday and on Monday, the mechanisms of energy balance control, which kick in then and try to increase food intake. You’re shaking your head. You don’t believe that? We can continue this discussion. Then, treatment of symptoms: I fully agree with you, but many of these reactions are originally designed to be defense reactions. At some point, if they are either too long or too strong, they can have negative consequences. The same holds for the anorexia, at some point if the anorexia just leads to a decrease in body weight that reaches a certain point, then this symptom by itself can have a negative effect. And in this case, it may make sense to treat the symptom, even if you cannot cure the disease. So, yes, I agree, it is just a treatment of symptoms, but there are situations in which you have to treat the symptoms. And then your last point was that illness is basically, if I understood you correctly, a transition where the body switches from the postabsorptive to the starvation state. I would argue about that, because disease cachexia is different from starvation. It’s different from starvation in the way that in disease cachexia, you may remember this last diagram that I showed, you have active mobilization of muscle tissue as well as lipolysis, whereas in starvation, you get less mobilization of protein. You don’t have in starvation these cytokine effects that lead to protein breakdown. So I think there is a difference.

**Dr. Kaiser Ahmed:** What have been the effects on the cardiovascular status and the electrolytes, while using calcium antagonists and other treatment modalities? This would be very important from the point of view of treatment, where once these treatment modalities are used in children?

**Dr. Wolfgang Langhans:** As I pointed out a little earlier, at least to the best of my knowledge these calcium antagonists have not been used in humans to treat anorexia. They have been used in clinical settings to counteract protein breakdown, and again this last diagram referred to that. There are various types of calcium antagonists, calcium channel-blockers, or calcium release-blockers such as danalone for instance, and they have been used. I have to admit right now that I’m not aware of the cardiovascular effects of these substances. I’m sure this is described, and I can give you the references of these papers that I’m referring to.

**Dr. John Mathai:** We’ve heard that pro-inflammatory cytokines mediate infection. It should follow that more severe infections would cause more severe anorexia, but in clinical practice we don’t find this. There are some infections that cause more anorexia and some infections that don’t. My question is why? Second question is pro-inflammatory cytokines, if I’m not mistaken, do have some beneficial role in infections. Is it a good idea to block pro-inflammatory cytokines just to increase the appetite?

**Dr. Wolfgang Langhans:** Could you please repeat, or let me answer the first question first and then repeat the second question, okay? So why is that different? I think I referred to that
a little bit earlier already when I said that different diseases activate a different pattern of pro-inflammatory and anti-inflammatory cytokines. Of course, what we are doing here is taking a simplistic view by just concentrating on the pro-inflammatory cytokines. As a matter of fact, when I mentioned these mRNA measurements in the brain in relation to cytokines, we did RNase protection assays for several components of cytokine systems, and there are marked differences in anti-inflammatory cytokines too. So the balance of pro- and anti-inflammatory cytokines ultimately determines the outcome, and this can be different and must not necessarily correlate with the severity of an infection. Of course it’s not just related to the infectious agent, but also to the location in the organism where the infection is. An infection of the intestine will have other effects than hepatitis or any other infectious disease.

Dr. John Mathai: The last question was about the beneficial role of these pro-inflammatory cytokines in the infections per se. So trying to block that, would it adversely affect the outcome from the infection? It may attenuate anorexia, but would it not influence the outcome of the infection per se?

Dr. Wolfgang Langhans: I see what you mean. It’s a sensitive balance and if you interfere with the cytokines, be it by trying to block pro-inflammatory cytokines or by enhancing anti-inflammatory cytokines, you always have to be aware that you will also affect the immune functions that are related to these cytokines, and I refer to that in my summary where I mention that for an attenuation of the anorexia it may be more promising to antagonize the neurochemical mediators of anorexia because this doesn’t carry the risk of interfering with immune functions with potentially beneficial effects.

Dr. Michael J. G. Farthing: We know from human studies and in clinical practice that patients who have severe sepsis or injury that are nutritionally supported, say through intravenous nutrition, that it’s very difficult to achieve positive nitrogen balance. You can just about achieve calorie balance by putting in lots of calories, but what you end up with is deposition of fat and not restoration of the somatic muscle. And I wonder whether there’s any evidence from either your acute or chronic models as to what happens to body composition, when these animals have their appetite restored or at least feeding is restored towards normal. So are you effectively putting fat on these animals or do they actually get a normal body composition?

Dr. Wolfgang Langhans: I’m not aware of any studies that address this interesting point. But the reason for the failure to achieve a positive nitrogen balance presumably is that by parenteral nutrition you do not affect this cascade that leads to protein breakdown.

Dr. Anne Ballinger: No, I don’t know of any, and we did not publish in this TNBS colitis model that when we gave the interleukin-1 receptor antagonist, we didn’t actually find that the body composition was the same as pair feeding in fact. So in relation to this thing about losing muscle mass, in TNBS colitis, as well as the body weight falling in parallel to pair fed, the body composition is the same for pair fed in either case they lose both protein and fat.

Dr. Michael J. G. Farthing: But it may be different in acute models, and I think obviously the concerns about these sorts of interventions are more worrying in the chronic situation.

Dr. S. Vasant Kumar: I was worried about these cytokine interventions, because throughout the world there has been an increase in 8-OB ostman hyperreactive diseases, because the cytokines block the TH-1 system. Has there been a switchover to the TH-2 system, with more triggering of cyclocstis pathways?

Dr. Wolfgang Langhans: Again, I agree with you. Interfering with cytokines always carries the risk that you also get “negative side effects,” because of the important immune functions of the cytokines. I fully agree. I don’t think there is any other answer to that.
Dr. Abdul Majid Molla: This is just a comment in response to what Professor Farthing was saying. Studies of nutritional rehabilitation of the severely malnourished children showed that the weight gain during the recovery phase is very high, as much as 20 or 30g/kg/day. In the first 12 to 18 weeks, there is more fat laid on the body and responsible for weight gain, and after 12 weeks, there seems to be a little bit less weight gain and there is a balance between the fat and the lean body mass. Initially there is a lot of fat put on.