

**A TRIBUTE TO DR. SAMUEL MCDONALD MCCANN'S LIFE
AS A SCIENTIST, MENTOR AND FRIEND.**

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INTRODUCTION.

We have had the honor and pleasure to have worked and shared a sincere and unique friendship with Dr. S.M. McCann. Dr. McCann was a unique person: enthusiastic, inspiring and a terrific scientist whose passion for science and knowledge were limitless. His scientific achievements as a pioneer of the two interrelated fields of neuroendocrinology and neuroimmunomodulation included more than 1,000 publications and a number of outstanding scientific prizes. Above all, Don was a generous, talented mentor and fostered the career of several young scientists, who have become world-renowned academic leaders in leading universities and research centers. This mini-review focuses on our collaborative work with Dr. McCann, providing evidence for a bidirectional communication between the neuroendocrine and -immune systems. Interestingly, it is believed that chronic stress can alter the balance between this bidirectional interaction and eventually cause psychiatric disorders (20).



*Professor Dr. Samuel McDonald McCann,
a pioneer in Neuroendocrinology
and Neuroimmunomodulation
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**NEUROENDOCRINE-IMMUNE INTERACTIONS: REGULATION OF LEPTIN BY
STRESS AND INTERACTIONS WITH NITRIC OXIDE AND TUMOR NECROSIS
FACTOR- α .**

Leptin, a hormone disguised as a cytokine.

Leptin, the first hormone found to be synthesized in adipose tissue, is a protein of 167 aa that has three-dimensional structure homology with cytokines that belong to class-I

family such as interleukin-6 (IL-6) (23). Moreover, the structure of the long form of leptin receptor (OB-Rb) is highly homologous to that of glycoprotein 130 (gp130), the common signal transducing receptor component for the IL-6 family. Leptin also shares functional relationships with elements of the immune system. In fact, *ob* mice show decreased immune response and are more sensitive to the lethal effects of tumor necrosis alpha (TNF- α) (18) and lipopolysaccharide (LPS) (3). As leptin has structural and functional similarities with cytokines and because the synthesis and release of these immune-related factors are altered by different types of stress, we have studied the neuroendocrine control of leptin during the inflammatory stress produced by LPS and its possible interaction with proinflammatory elements of the immune system such as nitric oxide (NO) and the powerful proinflammatory cytokine TNF- α .

Neuroendocrine control of leptin during inflammatory stress.

Administration of peripheral or central LPS into rodents mimics the changes observed during sepsis and systemic inflammatory immune response syndrome (SIRS). As leptin has both structural and functional homologies with several cytokines (24), we studied the effects of inflammatory stress triggered by LPS on leptin synthesis and release *in vivo* using animal models in which conscious male rat had indwelling jugular catheters. Our initial results showed that LPS evoked a rapid and long-lasting increase in plasma leptin concentrations with the first significant increase occurring within 10min and plateauing from 2 to 6 h. To determine if LPS-induced effects on leptin were neurally controlled, our first approach was to study the action of the anesthetic ketamine before administration of LPS (14). In previous studies we had shown that prior to the insertion of the jugular catheter, plasma leptin gradually declined after the administration of ketamine (11). Similarly, in animals bearing a jugular catheter, ketamine also decreased baseline plasma leptin concentrations during the first 2 h and surprisingly they rebounded later between 2-6 h. Moreover, ketamine also decreased LPS-induced increases in plasma leptin, supporting the concept that leptin synthesis and release are neurally controlled. We then studied the putative role of the sympathetic nervous system in the control of plasma leptin levels and synthesis in adipose tissue. To this end, we assessed the effects of α -adrenergic and β -adrenergic agonists and antagonists in the LPS response. Administration of a single injection of the β -adrenergic agonist isoproterenol slightly decreased plasma leptin concentrations, while in presence of LPS, isoproterenol largely blunted the LPS-induced increase of plasma leptin. The β -adrenergic antagonist propranolol only increased plasma leptin levels when it was injected alone, but in the presence of LPS it failed to significantly alter the LPS-induced increase of plasma leptin. Therefore, those results suggest that catecholaminergic input acting through β -adrenergic receptors is capable of inhibiting leptin release during resting conditions but not during inflammatory stress. We also examined the catecholaminergic action through alpha-adrenergic receptors by studying the action of phentolamine, an alpha-adrenergic antagonist. Phentolamine rapidly increased plasma leptin concentrations either when it was injected alone or in presence of LPS (14). Consequently, our results support the concept that catecholamines exert an inhibitory effect in plasma leptin levels through alpha-adrenergic receptors during both resting and stress conditions. Since the biological effect of phentolamine was more pronounced than that produced by propranolol, the catecholaminergic action through alpha-adrenergic receptors seems to prevail over that through β -adrenergic receptors.

There is more evidence suggesting that leptin is centrally regulated as we have characterized an ultradian rhythm for plasma leptin both in humans (7) and rats (10). Our search to understand which endocrine elements exert this central control led us to the re-

alization that prolactin (PRL), an hormone that displays a diurnal variation similar to that of leptin, exerts a tonic stimulation on leptin release (10). In fact administration of PRL in rats rapidly increased plasma leptin concentrations; whereas, α -bromoergocryptine, a D-2 receptor agonist that inhibits PRL release from the anterior pituitary gland, produced the opposite effect (10). The plasma concentration of PRL, was also increased by LPS in experimental paradigm similar to that mentioned before. It is therefore likely that LPS-induced leptin release is elicited by the activation of PRL receptors present in adipocytes. For this reason, we studied the effect of α -bromoergocryptine, an inhibitor of PRL release, alone or in the presence of LPS (10). Alpha-bromoergocryptine initially decreased plasma leptin concentrations, but this decrease was followed by a later rebound similar to that elicited by ketamine (10). We hypothesized that the rebound in both cases was related to an initial fall of PRL, which then triggered an inhibition of the tonical dopaminergic negative feedback of PRL release in the pituitary. Then, as the effect of the drug or anesthesia vanishes, there is a subsequent increase in PRL synthesis and release leading to leptin rebound. In summary, we hypothesize that one of the central actions exerted by LPS is the inhibition of the secretion of dopamine (DA), which in turn removes the inhibitory tone caused by tuberoinfundibullar dopaminergic neurons on the secretion of PRL. We also hypothesized that the increased of lactotropes' (L) secretion of PRL reaches the adipose tissue through the circulation and acts in the receptors (PRL_r) present in the adipocytes resulting in increased release of leptin stored in cytoplasm pinocytotic vesicles (14).

Our data support the concept that LPS increases leptin synthesis and release, at least in part, through increased release of PRL. In contrast, LPS-induced sympathetic activity leads to inhibition of leptin synthesis and release, an effect that is mainly driven through α and, to a lesser extent, β -adrenergic receptor activation.

Leptin modulates the synthesis and release of elements of the innate immune system.

Previous studies have shown that the central effects exerted by leptin on LHRH release were mediated by increased synthesis and release of NO (21, 22). Nitric oxide is rapidly metabolized to nitrite [NO₂] and nitrate [NO₃]; two metabolites that diffuse into the circulation and serve as an index of NO production. Besides acting as a gaseous neurotransmitter, NO synthesis is also highly increased during the oxidative stress elicited by LPS-induced proinflammatory cytokines (15, 19). In contrast, during baseline conditions peripheral NO, synthesized mainly from endothelial NOS3, exerts physiological actions controlling blood flow and blood pressure. Leptin displays structural and functional similarities with different cytokines (23) and it has detectable blood levels during resting conditions. The major storehouse of leptin is the adipocyte, this cell type that has been described to express NOS3 and the long, biologically active, isoform of leptin receptor (OBRb) on their cell membrane. We hypothesized that leptin could exert autocrine and paracrine actions through receptors located in adipocyte membrane and adjacent capillary endothelial cells, activating NOS3 and increasing NO to cause vasodilation and increased blood flow and thus controlling peripheral synthesis and release of NO.

The fact that leptin displays an ultradian variation led us to study if a diurnal correlation between plasma leptin and NO₃-NO₂, as an index of NO production, existed in the rat. Our results indicated that plasma NO₃-NO₂ concentrations have circadian variations and that those concentrations were correlated throughout the 24h with plasma leptin levels, which suggest that one of these factors controls the other (12). Because LPS-induced increase of plasma leptin concentration was not mediated by NO (13), we hypothesized that leptin would control NO synthesis. Therefore, we determined the effect of leptin on NO₃-NO₂

by performing *in-vitro* and *in-vivo* experiments. Our *in-vitro* experiments were designed to investigate the putative role of leptin on its own cell source namely, the adipocyte. In fact, the adipocyte, is a cell type that has leptin receptors on its cellular membranes, expresses both the inducible NOS (NOS2) and the constitutive endothelial NOS3 isoforms. We tested the effect of leptin within the range 10^{-9} to 10^{-5} M in epididymal fat pads incubated *in-vitro*. The highest dose of leptin (10^{-5} M) increased $\text{NO}_3\text{-NO}_2$ release in the tissue culture medium indicating, that leptin directly or indirectly activated NOS causing release of NO and supporting the concept that leptin-induced NO would cause vasodilation by diffusing to adjacent arterioles and venules to relax their smooth muscle via activation of guanylyl cyclase and generation of cyclic guanosine monophosphate (cGMP) from guanosine-5'-triphosphate (GTP) (12). Surprisingly, a lower concentration of leptin than the one that showed to be effective to increase NO was capable of increasing TNF- α 4-fold, and the highest concentration of leptin (10^{-5} M) increased TNF- α 60-fold. Considering the fact that TNF- α is a strong releaser of NO, the possibility exists that leptin-induced TNF- α might activate NOS resulting in increased release of NO. All of these *in-vitro* results were confirmed *in-vivo*, in conscious rats bearing a jugular catheter (12).

In summary, these results support the concept that cytokines can be regulated in a neuroendocrine manner. Indeed, leptin is not only regulated by the sympathetic nervous system and PRL but it also interacts and regulates proinflammatory immune elements such as NO and TNF- α .

IMMUNE TO NEURAL INTERACTIONS: A CNS INFLAMMATORY CASCADE ORCHESTRATED BY INTERLEUKIN-1 BETA (IL-1 β).

The dysregulation of proinflammatory cytokines such as IL-1 β may constitute one of the major factors underlying neurodegenerative and psychiatric-related diseases such as Alzheimer's and major depressive disorders (MDD) (2, 8, 16). There is interesting evidence showing that different classes of antidepressants decrease the synthesis and release of major proinflammatory elements such as IL-1 β (1) and the activity of cyclooxygenase-2 (COX-2) (17) suggesting that normalization of components of the innate immune system might constitute a prerequisite to accomplish symptom remission. The fact that 30-45% of the patients develop MDD during interferon-alpha (IFN- α) therapy for viral hepatitis and several malignancies, supports a causative relationship between proinflammatory cytokines and neuropsychiatric disorders. In animal studies it was recently demonstrated that administration of IFN- α decreased hippocampal neurogenesis by a mechanism that required IL-1 β (5), suggesting that IL-1 β could be implicated in some of the adverse neuropsychiatric side effects described above. In order to better understand the immune to neural communication and the molecular events that are orchestrated within the brain by IL-1 β , we have employed an animal model of LPS-induced SIRS, a pathophysiological condition that only in the US causes the loss of more than 200,000 lives/year (9). The crucial role of IL-1 in inflammation has been highlighted by studies performed in caspase-1 knockout mice (casp1 $^{-/-}$): these transgenic mice lack mature IL-1 β and synthesize lower amounts of IL-1 α ; they are resilient to lethal doses of LPS. The facts that IL-1 β expression is spatio-temporally regulated within the brain and that many of the actions exerted by LPS and/or proinflammatory cytokines within the CNS are exerted at doses that are not effective in the periphery led us to hypothesize that central increase of IL-1 β expression could orchestrate a central inflammatory cascade which dysregulation could contribute to the fatal outcome after administration of a lethal dose of LPS in wild-type (WT) mice. To this end, we compared the brain gene expression of wild-type and casp1 $^{-/-}$ after 6h of administration of saline or LPS. During the confir-

mation studies using real-time PCR (Polymerase chain reaction), we found that eight genes displayed lower level of LPS-induced expression in casp1^{-/-} mice compared to WT mice. These results suggested that IL-1 β is a major factor that controls the expression of those genes during SIRS. These genes were two guanosine triphosphate hydrolase, (GTPases) (TGTP, T-cell specific GTPase, and GBP-2, guanilate binding protein 2, interferon inducible), two chemokines (chemokine (C-X-C motif) ligand 1 (CXCL-1) and CXCL-10), the metalloprotease ADAMTS1 (a disintegrin and metalloproteinase with thrombospondin type 1 motif, 1), interleukin-1 receptor antagonist (IL-1RA), NOS2 and COX-2 (9). Our results revealed the relevant role of biologically active IL-1 β in the expression of all of the mentioned genes which might be functionally related a cascade mechanism. It is well-known that NO increases the synthesis of PGs by stimulating the expression of the inducible COX-2. It appears that the lower stimulation of the DNA-directed synthesis of NOS2 in casp1^{-/-} mice led to decreased expression of COX-2. Chemokines play a crucial role in the recruitment of mononuclear cells towards the inflammatory site. The decreased level of expression of CXCL-1 and CXCL-10 in casp1^{-/-} mice during the LPS-induced inflammatory reaction might therefore account for decreased recruitment of monocytes and neutrophils within the brain. Regarding ADAMTS1, the diminished expression of this metalloprotease could also be neuroprotective, since metalloproteases were described to increase the blood-brain barrier (BBB) permeability during inflammation. These results taken together suggest that the casp1^{-/-} mice CNS is more refractory to the deleterious effects of LPS-induced SIRS and could thus explain, at least in part, the survival of casp1^{-/-} and transgenic mice overexpressing IL-1RA to lethal doses of LPS (4, 6).

CONCLUSIONS.

We dedicate this modest tribute to Dr. S.M McCann's genius as one of the greatest pioneers in neuroendocrinology. Above all, he was an inspirational individual who has greatly influenced several generations of scientists. His pioneering work was not only limited to the basic action of hormones and cytokines. On the contrary, his original research, ideas and concepts should be interpreted in a broader frame. Indeed, it is unquestionable that his contributions elucidating the bidirectional communication between the neuroendocrine and immune systems has paved the way to understand the imbalance in this bidirectional communications that seems to underlie neuropsychiatric conditions, including major depressive disorder, a pathological condition that affects millions of patients and is the number one cause of suicide. We have been privileged and feel blessed to have worked and shared a special friendship with Don. We certainly owe him an enormous debt of gratitude for his genius and life-long incessant pursuit of scientific knowledge. A take home message is the example of Don McCann's passionate, intelligent and honest search for scientific truth. At a time when science has become increasingly politicized it is important to keep in mind the path of Don's selfless commitment to advance science, which ought to serve as an inspiration to all of us.

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