Masterclass in Neuroendocrinology 13



Jan Pieter Konsman
Teresa M. Reyes *Editors*

Neuroendocrine-Immune System Interactions

International Neuroendocrine Federation

Springer

Neuropeptide Binding Autoantibodies Regulating Neuroendocrine Communications

8

Serguei O. Fetissov and Mouna El Mehdi

Abstract

Immunoglobulin (Ig) molecules or antibodies binding neuropeptides or peptide hormones that regulate motivated behavior, stress, and emotion, such as α-MSH, ACTH, oxytocin, or ghrelin, are naturally present in humans and in rodents in both healthy and disease conditions. The functional role of such autoantibodies was analyzed, showing their constitutive participation as modulators of peptidergic signaling in the neuroendocrine system. In particular, IgG were shown to play a role of peptide carriers and allosteric modulators of peptide receptors. Moreover, the pathway of discovery of the origin of α-MSH-reactive autoantibodies leads to the identification of a specific bacterial protein, ClpB, as an antigen mimetic of α-MSH, a key anorexigenic peptide. This discovery contributed to a better understanding of the role of the immune system in neuroendocrine communication between gut microbiota and the host and was further used for the development of a new generation of probiotics to control appetite. Furthermore, experimental data showed that an autoimmune reaction against ClpB, leading to the production of cross-reactive pathogenic α-MSH-reactive autoantibodies, may underlie the origin of eating disorders. Thus, in this chapter, we show that the characteristics of autoantibodies reacting with peptide hormones may provide valuable data that help to better understand the molecular mechanisms of neuroendocrine communication and to create therapeutic approaches for altered motivated behavior, response to stress and emotions.

S. O. Fetissov (✉) · M. El Mehdi
Neuroendocrine, Endocrine and Germinal Differentiation and Communication Laboratory, Inserm UMR1239, University of Rouen Normandy, Mont-Saint-Aignan, France
e-mail: Serguei.Fetissov@univ-rouen.fr

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2023

J. P. Konsman, T. M. Reyes (eds.), *Neuroendocrine-Immune System Interactions*, Masterclass in Neuroendocrinology 13,
https://doi.org/10.1007/978-3-031-21358-8_8

Keywords

Neuropeptides · α-MSH · Oxytocin · ACTH, ghrelin · ClpB · Peptide hormones · Autoantibodies · Immunoglobulins · Motivated behavior · Stress · Feeding behavior · Appetite · Social behavior · Psychoneuroimmunology · Hypothalamo-pituitary-adrenal axis · Molecular mimicry · Gut microbiota-brain axis

Neuroendocrine system and immune system interactions, for the Masterclass in Neuroendocrinology Series

8.1 Introduction. Peptide Signaling in the Neuroendocrine System

Peptide hormones and neuropeptides are the main signaling molecules in the neuroendocrine system. Typically, small proteins of less than 100 amino acids are called peptides, but larger protein molecules may play roles similar to peptides in neuroendocrine signaling. Peptides are co-produced and co-released with a number of classical transmitters and combinations of both represent a chemical signature of a specific group of neurons or endocrine cells involved in a more or less specific function (Hökfelt et al. 2018). The distinction between peptide hormones and neuropeptides is more terminological than functional, since both can be produced and may act in neuronal and non-neuronal tissues; for example α-melanocyte stimulating hormone (α-MSH) is a neuropeptide produced in the brain and a hormone produced in the pituitary as well as a paracrine factor in some peripheral tissues, such as skin. In this chapter, the terms neuropeptide and peptide hormone will be used interchangeably.

The immune system has multiple ways to influence neuroendocrine signaling, and the present chapter is focused on the role of naturally occurring autoantibodies, i.e., immunoglobulins (Ig), reacting with some peptide hormones having well-known functions in the neuroendocrine system. IgG and other classes of Ig are most abundant in the blood, and therefore the discussion of their interactions with peptide hormones will primarily concern peripheral circulation. Indeed, IgG represent about 80% of all immunoglobulins in human blood, where they are at levels of 70 μM (10–20 mg/ml), second only to albumin (Wang et al. 2008; Hortin et al. 2008). IgG are also present in the central nervous system (CNS) at concentrations of 0.1–0.2 μM, i.e., approximately 500 times lower than in the systemic circulation and accounting for about 8% of the total protein in the cerebrospinal fluid (CSF) of adult humans (Davson and Segal 1996). A situation opposite to that of IgG occurs with neuropeptide hormones preferentially produced in the brain, such as oxytocin and vasopressin, with extremely low concentrations in the blood (~1–10 pg/ml) (Leng and Ludwig 2008). Nevertheless, systemic action of both central and peripheral neuropeptides is of physiological importance, and hence, the mechanisms regulating

peripheral peptide signaling are of practical relevance. Moreover, since IgG are present in the CNS, we cannot exclude their central action in modulating neuropeptide signaling.

Beside their signaling properties, peptides are also a source of cellular nutrition, and undergo degradation within a few minutes by plasma and membrane-bound enzymes such as dipeptidyl peptidase (DPP). The only way for peptides to escape degradation is to be bound to a larger carrier molecule that will protect the peptide until it binds to its specific receptor. Only a few peptides, such as corticotropin-releasing hormone (CRH), have specific binding proteins (Ketchesin et al. 2017). Peptide stability is a key issue of their biological activity *in vivo,* both under natural conditions and when peptides are used as drugs. As will be discuss below, it is possible that IgG may improve peptide stability by playing the role of carrier molecules.

The biological action of peptides as messengers in the neuroendocrine systems occurs upon their binding to specific receptors, which are typically G-protein coupled receptors (GPCR). Activation of GPCR by peptides is modulated by endogenous allosteric modulators, e.g., activation of the melanocortin 4 receptor (MC4R) by α-MSH is enhanced by its co-binding of Ca^{2+} (Yu et al. 2020). As discussed below, IgG may also play a role as an allosteric modulator of MC4R, which is relevant to obesity and eating disorders.

8.2 Detection of Neuropeptide-Reactive Autoantibodies: Methodology

The term autoimmune refers to a self-reaction of the immune system. However, the term autoantibody does not automatically imply a pathological reaction leading to tissue damage by immune cells, which is present in autoimmune disorders such as diabetes type 1. In fact, many so-called natural autoantibodies are present in healthy individuals as a part of the total Ig pool (Lacroix-Desmazes et al. 1998). Table 8.1 summarizes the majority of studies showing the detection and analysis of autoantibodies reacting with various neuropeptides and peptide hormones in humans and rodents in health and disease. Autoantibodies of classes IgM, IgA and IgG have been detected by several techniques. For instance, the binding of plasma IgG to neuropeptides on brain sections has been visualized using immunohistochemistry. This technique is also useful for screening of human plasma for the presence of selective staining which can be further explored for the detection of binding antigens. For instance, it was used for the identification of IgG reactive with α-MSH in the plasma of patients with eating disorders (Fetissov et al. 2002) (Fig. 8.1).

Most commonly, plasma levels of neuropeptide-reactive autoantibodies of various Ig classes are assayed by enzyme-linked immunosorbent assay (ELISA). A technical protocol for neuropeptide autoantibody detection by ELISA is available (Fetissov 2011). The protocol allows the analysis of both the "free" and the "total" fraction of IgG autoantibodies, by incubating the plasma or serum samples in normal

Table 8.1 Occurrence of autoantibodies reactive with neuropeptides/peptide hormones

Neuropeptide/ Peptide hormone-reactive autoantibodies in plasma	Species	Methods of detection	Clinical relevance	Reference
α-MSH, IgG, IgM, IgA	Hu, Rt, Mo	ELISA, IHC, SPR, RBI	Eating disorders	Fetissov et al. (2002, 2005, 2006, 2008), Hamze Sinno et al. (2009), Coquerel et al. (2012), Lucas et al. (2019), Tennoune et al. (2014, 2015), Molfino et al. (2020), Karaiskos et al. (2010), Wallenius et al. (2019), Roubalova et al. (2021)
Galanin and α-MSH IgG in both plasma & CSF	Hu, Rt	ELISA	Alzheimer's disease	Costa et al. (2011), Fetissov et al. (2008)
ACTH IgG, IgM	Hu, Rt	ELISA	Aggressive behavior	Fetissov et al. (2002, 2005, 2006, 2008), Værøy et al. (2018), Tennoune et al. (2015), Karaiskos et al. (2010), Schaefer et al. (2013)
Oxytocin IgG, IgM	Hu, Rt	ELISA	Depression, eating disorders	Fetissov et al. (2006), Karaiskos et al. (2010), Garcia et al. (2011)
Vasopressin IgG, IgM	Hu, Rt	ELISA	Depression, eating disorders	Fetissov et al. (2006), Karaiskos et al. (2010), Garcia et al. (2011)
NPY, IgG, IgM, IgA	Hu, Rt	ELISA	Depression	Karaiskos et al. (2010), Garcia et al. (2012)
CRH, IgG, IgA	Hu, Rt	ELISA	Psychopathological traits	Karaiskos et al. (2010), Fetissov et al. (2008)
Ghrelin	Hu, Mo	ELISA, SPR	Obesity, anorexia, stress, rheumatoid arthritis	Takagi et al. (2013), Terashi et al. (2011), François et al. (2015, 2016), Porchas-Quijada et al. (2019), Espinoza-Garcia et al. (2021)
Orexin/ hypocretin IgG, IgM, IgA	Hu	ELISA, RBI	Narcolepsy	Deloumeau et al. (2010), Tanaka et al. (2006), Black et al. (2005), Wallenius et al. (2019)
Leptin, IgG, IgA	Hu, Rt	ELISA, SPR	Obesity, diabetes	Bouhajja et al. (2018), Fetissov et al. (2008), Espinoza-Garcia et al. (2021)

(continued)

Table 8.1 (continued)

Neuropeptide/ Peptide hormone-reactive autoantibodies in plasma	Species	Methods of detection	Clinical relevance	Reference
Peptide YY, IgG, IgA	Hu, Rt	ELISA	Not studied	Fetissov et al. (2008)
Agouti-related protein, IgG, IgA	Hu, Rt	ELISA	Not studied	Fetissov et al. (2008)
Melanin-concentrating hormone, IgG, IgA	Hu, RT	ELISA	Not studied	Fetissov et al. (2008)
Vasoactive intestinal peptide (VIP) IgG	Hu	ELISA	Asthma, muscular exercise	Paul et al. (1985, 1989), Paul and Said (1988)
Bradykinin, IgG	Hu	ELISA	Alzheimer's disease	Myagkova et al. (2003)

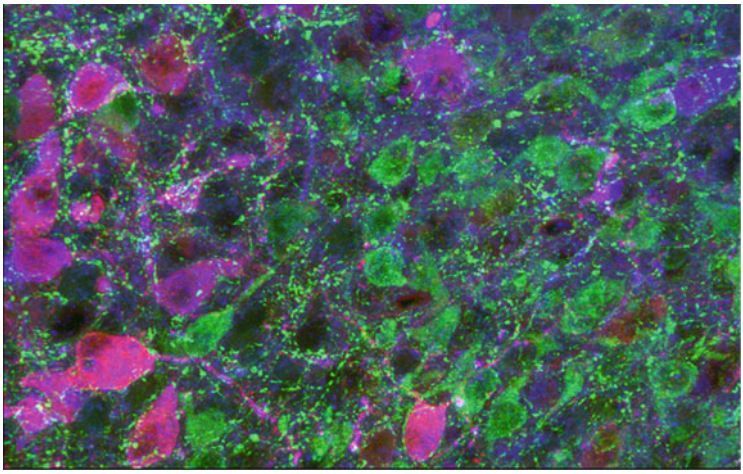

Fig. 8.1 Immunohistochemical visualization of plasma IgG from a patient with bulimia nervosa in the arcuate nucleus of the hypothalamus in a rat brain section. The patient's plasma contains α-MSH-reactive IgG, which binds to anorexigenic proopiomelanocortin neurons (pink). The neighboring population of orexigenic NPY/AgRP neurons was stained with commercial rabbit NPY antisera (green). Confocal fluorescence image by Serguei O. Fetissov

or dissociative buffers, respectively. Such an approach may provide complementary data on the relative levels of "free" and "complexed" antibodies, which may reflect altered peptidergic signaling in some pathological conditions. For instance, changes of plasma levels of free and total fractions of IgG autoantibodies reactive with adrenocorticotropic hormone (ACTH) in humans with various levels of aggressive behavior showed that their relative ratio was increased in the group of most

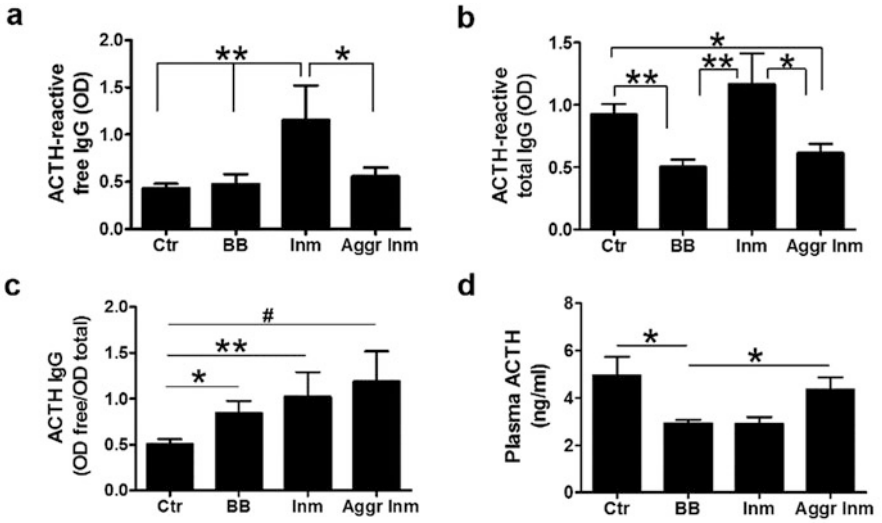

Fig. 8.2 Plasma levels of free (**a**) and total (**b**) ACTH-reactive IgG, their ratios (**c**) and plasma concentrations of ACTH peptide (**d**) in subjects with various degree of aggressive behavior. *Ctr* non-aggressive controls, *BB* body builders, *Inm* inmates, *Aggr inm* inmates committed violent aggression. Figure from Vaeroy et al. (2018)

aggressive subjects and was correlated negatively with the plasma concentration of ACTH peptide (Fig. 8.2). Such data indicate the role of plasma IgG as modulators of peptidergic signaling in the mechanism of stress-response and in stress-related disorders (Vaeroy et al. 2019). The role of ACTH-reactive IgG in modulating ACTH-induced cortisol secretion is discussed below.

Other techniques of peptide hormone reactive autoantibodies detection, such as the liquid-phase radiobinding assay (RBA), are of clinical relevance to classic autoimmune diseases (Sebriakova and Little 1973). For instance, it is used for analysis of the plasma levels of anti-insulin antibodies in type 1 diabetes, which is often performed in insulin-treated patients (Demeester et al. 2015). Because of the abundant literature on anti-insulin autoantibodies in the autoimmune disease context, we did not include them in Table 8.1. Nevertheless, insulin-reactive IgG can be present at various levels in non-insulin-treated subjects and in other species, which may also classify them as natural peptide-reactive autoantibodies (Sodoyez et al. 1990; Nishii et al. 2010). In fact, the recognition of insulin by germinal B-cells supports the presence of natural insulin-like autoimmunity (Wan et al. 2016). Naturally occurring insulin autoantibodies may possibly modulate glucose metabolism, for instance, it was shown that the administration of anti-insulin antibodies in the portal vein influenced feeding behavior (Surina-Baumgartner et al. 1995).

Because the IgG class of antibodies typically undergo affinity maturation, the affinity kinetics of IgG interaction with neuropeptides can be an important property that can be characterized. For instance, increased affinity of insulin autoantibody was shown to be associated with diabetes type 1 (Achenbach et al. 2007). The affinity

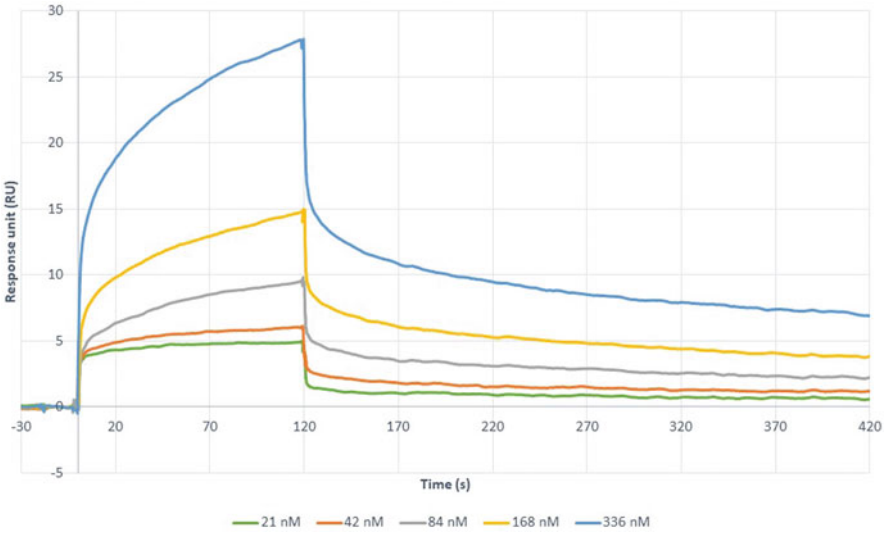

Fig. 8.3 SPR sensorgram obtained using a BIAcore T200 instrument for the interaction of the oxytocin peptide with serial dilutions of IgG (shown in different colors) purified from a healthy human plasma sample. Analysis of affinity kinetics shows that the equilibrium constant of this interaction (K_D) is 1.31×10^{-7} M. Image by Emilie Lahaye, Inserm UMR1239

kinetics can be analyzed using competitive RBA, typically used for the analysis of anti-insulin autoantibodies, or by the direct analysis of protein-protein interactions using the surface plasmon resonance (SPR) technique (Fig. 8.3).

8.3 Occurrence of Neuropeptide-Reactive Autoantibodies

Occurrence of autoantibodies reactive with several neuropeptides/peptide hormones in the plasma of humans and rodents has been reported in numerous studies (Table 8.1). It is possible that such occurrence may reflect a general phenomenon of immunoglobulin binding to peptidergic messengers involved in the neuroendocrine and other types of communication, e.g., in the immune system. In fact, autoantibodies reactive with cytokines have been detected in human plasma (Bendtzen et al. 1998). Like any Ig, neuropeptide-reactive autoantibodies are produced by B-cells and secreted into the systemic circulation, where they are usually detected in plasma or serum samples. For instance, the levels of IgG-reactive to ghrelin, the hormone of hunger produced in the stomach, is about 20-fold higher in plasma than in the hypothalamus or liver (Takagi et al. 2013).

The gastro-intestinal tract-associated lymphoid system is the largest part of the immune system in the body that may significantly contribute to the production of neuropeptide-reactive autoantibodies. In fact, gastric electrical stimulation was shown to activate c-fos marker in the gastric mucosal and submucosal layers and

increase plasma levels of both ghrelin and ghrelin-reactive IgG (Gallas et al. 2011; Gallas and Fetissov 2011). The vicinity of the gut lymphoid system to the gut microbiota may serve as a natural antigenic stimulus for the production of neuropeptide-cross reactive autoantibodies. Therefore, the gut is probably another organ where neuropeptide-reactive autoantibodies can be readily detected. In the cerebrospinal fluid (CSF), the neuropeptide-reactive IgG can be detected at low levels, reflecting their limited passage across the blood–brain barrier (BBB). For instance, galanin-reactive IgG, possibly transporting galanin in immune complexes, were detected in human CSF, where their levels correlated negatively with the severity of cognitive impairment, suggesting a functional relevance of such IgG to Alzheimer's disease (Costa et al. 2011). The possible role of IgG as neuropeptide carriers to the brain is discussed below in more detail.

When analyzing plasma neuropeptide autoantibodies in some pathological conditions, it may be found that some patients display lower levels than healthy controls. Such results could be interpreted by some scientists as "negative," i.e., as showing the absence of such autoantibodies. However, considering the physiological role of neuropeptide-reactive IgG, such a decrease may reflect pathological changes relevant to the disease. For instance, lower levels of orexin-reactive IgG found in patients with narcolepsy (Black et al. 2005; Deloumeau et al. 2010) reflected only the free fraction of circulating autoantibody, while most of orexin-reactive-IgG in narcoleptic patients was present in the form of immune complexes (Deloumeau et al. 2010). Nevertheless, the relevance of IgG complexes with orexin to the pathophysiology of narcolepsy is still unknown and the search for the mechanism of orexin neuron-directed autoimmunity continues (Mahoney et al. 2019).

8.4 Role of Autoantibodies as Peptide Carriers

The function of immunoglobulins as carrier molecules of peptide hormones serves to improve peptide stability. Such a function was revealed by studying the peptide ghrelin, which is highly unstable in the circulation, where it is deacylated by plasma enzymes leading to the loss of ghrelin's orexigenic effect. Preincubation of ghrelin with IgG protected the hormone from deacylation, while the absence of IgG led to a complete loss of the acylated form. Importantly, the presence of ghrelin in the immune complex with IgG preserved its biological effect of stimulating food intake (Takagi et al. 2013). Furthermore, using chromatographic purification of IgG from human plasma, the same study showed that a large amount of the total pool of circulating ghrelin is present bound to IgG, further supporting the IgG carrier role for this peptide hormone. Moreover, while about half of the total ghrelin was found as the bound form in healthy subjects, in obese patients it was 4 times higher, which could be due to an increased affinity of IgG for ghrelin (Takagi et al. 2013). The phenomenon of increased carrier function of ghrelin by IgG may explain the enhanced orexigenic effects of exogenous ghrelin in obesity vs. non-obese subjects (Druce et al. 2005).

Another example, using a similar approach of peptide detection after IgG extraction from plasma, showed that IgG may serve as a carrier molecule for nerve growth factor in both healthy humans and in patients with autoimmune disorders (Dicou and Nerriere 1997). A protective effect of IgG for leptin was suggested by significant correlations between increased IgG binding to leptin and lower body mass index in healthy subjects, and inversely, decreased affinity of IgG for leptin in obesity (Bouhajja et al. 2018). As mentioned above, plasma ACTH correlates with ACTH-reactive IgG levels. Taken together, these data suggest that the main biological role of neuropeptide-reactive IgG autoantibodies is to serve as peptide carriers. This role can be exploited to improve peptide hormone stability after their pharmacological administration. Such a possibility was supported by the demonstration of the enhanced pharmacological effects of ghrelin combined with IgG in the alleviation of activity-based anorexia in mice (Legrand et al. 2016).

8.5 Role of Autoantibodies in Neuropeptide Receptor Activation (α-MSH, ACTH, Oxytocin)

Here we discuss only the effects of IgG in immune complexes with neuropeptides in activation of the corresponding neuropeptide receptors; this should not be confused with anti-receptor antibodies, which can be relevant to neuroendocrine signaling but constitute a different topic. As discussed above, the immune complex of IgG with ghrelin preserved the peptide's biological activity, suggesting that such a complex could bind and activate the corresponding peptide receptor. Indeed, an *in vitro* study indicated that ghrelin affinity of binding to its receptor GHS-R1a was increased when ghrelin was present as an immune complex with IgG from obese subjects (unpublished).

More detailed data of the IgG modulatory role in neuropeptide signaling were obtained for α-MSH binding to human MC4R (Lucas et al. 2019). Using a cAMP assay, it was found that IgG decreased the threshold of receptor activation and that this effect of IgG was not present in obesity. Moreover, using *in vitro* microscopy, it was shown that the α-MSH/IgG immune complex binds to the MC4R on the cell membrane and is then internalized, reflecting receptor activation. Of relevance to the critical role of MC4R activation in decreasing food intake, the internalization rate of α-MSH/IgG immune complexes was decreased in obesity but increased in anorexia nervosa. Analysis by epitope mapping of α-MSH binding to IgG suggested that the C-terminal of the α-MSH peptide needs to be available for MC4R activation, while its masking by IgG, for example in obesity, blocks the activation (Lucas et al. 2019).

The importance of binding by natural IgG to the different parts of a peptide hormone in modulating its biological activity has also been shown for the adrenocorticotropic hormone (ACTH) (Værøy et al. 2018). In fact, the predominant binding of IgG to the central part of 39 amino acid-long ACTH was associated with the preservation of ACTH-induced cortisol secretion by human cortical adrenal cells *in vitro*. In contrast, IgG binding to the N-terminal, which was more frequently observed in aggressive subjects, was associated with deficient cortisol secretion.

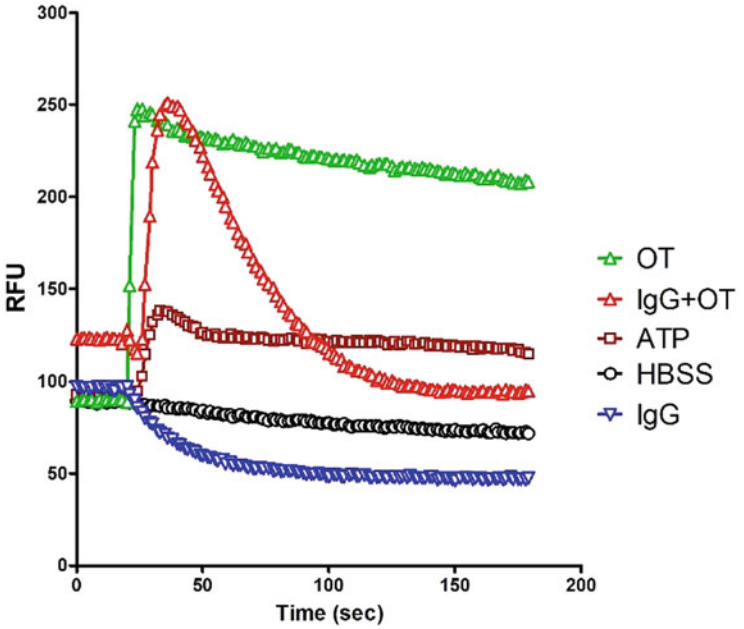

Fig. 8.4 IgG modulation of oxytocin-induced Ca^{2+} release by HEK293 cells expressing human oxytocin receptors. OT, oxytocin, ATP, adenosine triphosphate, HBSS, incubation buffer. IgG, example of IgG purified from plasma of a healthy subject. The area under the curve for oxytocin/IgG complex varies in different subjects (not shown), suggesting individual effects of IgG in complex with oxytocin in activation of oxytocin receptors. Data from the poster presentation of Lahaye et al. (2021)

A recent study showed that activation of the human oxytocin receptor, as assayed by Ca^{2+} release, can be modulated by plasma IgG in healthy subjects. As compared to oxytocin alone, oxytocin/IgG immune complex reduced the duration of Ca^{2+} release in all subjects. Furthermore, the amplitude of oxytocin/IgG complex-stimulated Ca^{2+} release varied in different subjects, sometimes being as high as after stimulation by oxytocin alone (Lahaye et al. 2021) (Fig. 8.4). Thus, the modulatory effects of IgG on neuropeptide-induced receptor activation suggests that naturally present IgG may contribute to the mechanism of individual variability in neuroendocrine signaling.

8.6 Catalytic Effect of Neuropeptide-Reactive IgG

It was found that IgG autoantibodies purified from a human plasma sample produced catalytic hydrolysis of vasoactive intestinal peptide (VIP), splitting it into two fragments (Paul et al. 1995). VIP, a 28-amino acid peptide from the glucagon/secretin family, stimulates vasodilation, suggesting a pathogenic role of neutralizing VIP IgG in some diseases such as asthma (Paul et al. 1989). The functional relevance

of the proteolytic IgG activity is probably dependent on the unique IgG structure and is a part of a more general catalytic activity of some IgG, concerning not only proteins and peptides but also DNA and RNA (Nevinsky and Buneva 2002). In fact, natural autoantibodies reactive to VIP are present in both healthy subjects and in patients with various autoimmune disorders (Bangale et al. 2002). It cannot be excluded that some catalytic IgG may occur naturally and hydrolyze peptide hormones and neuropeptides other than VIP, contributing to individual biological activity of neuroendocrine signaling in health and disease.

8.7 Peptide-Autoantibody Complex Penetration Across the Blood–Brain Barrier

IgG passes across the blood–brain barrier by saturable transport (Zlokovic et al. 1990); however, several key questions remain regarding the mechanisms involved. For instance, the rapid perivascular transport called the "glymphatic system" has been suggested to play a role in the spread of IgG once it has crossed the blood–brain barrier (Iliff et al. 2012). Another study suggested that the majority of systemically derived IgG in the brain is sequestered within the endothelial cell compartment (St-Amour et al. 2013). IgG antibodies possess two antigen-binding domains (Fab) for binding unique epitopes and a single fragment crystallizable domain (Fc) for the binding of cell surface receptors and the recruitment of effector functions. Known Fc receptors include the neonatal Fc receptor (FcRn or Brambell receptor) and Fc receptors specific for Ig isoforms (e.g., FcγR for IgG). Several studies showed that FcRn mediated the transport of IgG across peripheral vascular cells in both directions, while in the CNS the transport occurs mainly in the brain-to-blood direction (Roopenian et al. 2003; Schlachetzki et al. 2002). Nevertheless, the role of Fc and FcRn in the transport of IgG in the CNS remain controversial, and other mechanisms involving antigen receptor binding at the brain endothelial blood vessels, for example to transferrin and insulin receptors, have been discussed (Kouhi et al. 2021). Moreover, low-affinity binding to transferrin receptors may be necessary for the transport of IgG into the CNS (Bien-Ly et al. 2014). Penetration of the brain by neuropeptides in immune complexes with IgG still needs to be clarified, a preliminary study showed the presence of IgG in several brain structures involved in the barrier function after peripheral injection in the rat of α-MSH/IgG immune complexes (Fig. 8.5).

8.8 Antigenic Origin and Discovery Pathway to New Drugs

The search for the antigenic origin of α-MSH-reactive autoantibodies led to the identification of a bacterial mimetic protein of α-MSH, represented by caseinolytic protease B (ClpB) (Tennoune et al. 2014). Indeed, either active immunization of mice with ClpB or their gavage with ClpB-producing *Escherichia coli* (*E. coli*) K12 bacteria resulted in increased levels of anti-ClpB α-MSH-cross-reactive antibodies.

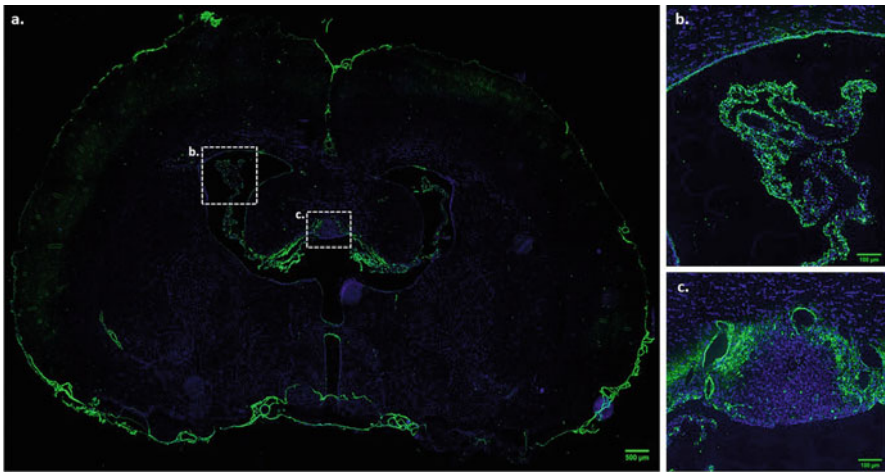

Fig. 8.5 Immunohistochemical detection of rat IgG in the rat brain 1 min after intracardiac injection of α-MSH/IgG immune complexes. (**a**) Coronal brain section at the level −0.8 mm from Bregma. Immunopositive structures (green) include: leptomeninges, choroid plexus, ventricular ependyma and the subcortical organ. Inserts (**b**) and (**c**) are shown with higher magnification, illustrating the choroid plexus and ventricular ependyma in (**b**) and the subfornical organ in (**c**) Nuclear counterstaining with DAPI (blue). Fluorescence image by Mouna El Mehdi

Importantly, when mice received a ClpB mutant *E. coli* strain, no increase in such antibodies was noticed. Indeed, the protein sequence of ClpB was found to contain an α-MSH-like fragment which is conserved in the ClpB molecule produced by a few bacterial taxa, including the whole family of Enterobacteriaceae. This finding suggests that a specific neuroendocrine signaling (ex. the melanocortin system) may be functionally connected to specific gut microorganisms (ex. Enterobacteriaceae), based on molecular mimicry between neuropeptides and bacterial proteins synthetized by such microorganisms (Fetissov 2019).

The discovery of bacteria producing an α-MSH-like anorexigenic protein led to a better understanding of the role of gut microbiota in the regulation of appetite and triggered a new line of research aimed at the development of ClpB-based probiotics for controlling appetite and body weight (Fetissov 2017). The proof of concept preclinical study was successfully accomplished in obese mice using a food-grade *Hafnia alvei* strain that expressed ClpB with an α-MSH-like epitope, similar to *E. coli* (Legrand et al. 2020). A follow-up study in overweight subjects confirmed the clinical efficacy of *H. alvei* by increasing a feeling of satiety and in reducing body weight and hip circumference (Déchelotte et al. 2021). Therefore, the discovery of α-MSH autoantibodies has served as a starting point for the development of a new generation of precision probiotics that can be further developed into biopharmaceutical drugs. Considering multiple examples of molecular mimicry between several neuropeptides and microbial proteins produced by commensal and pathogenic microorganisms, as well as the natural presence of neuropeptide-reactive Ig (Table 8.1), one may suggest that such autoantibodies have been induced by

homologous antigens derived from gut microbiota. Several research projects are ongoing to identify such antigens. Increased prevalence of some neuropeptide-reactive autoantibodies have also been found in *Helicobacter pylori* and *Candida albicans*-positive children and their relevance to short stature has been discussed (Stawerska et al. 2015).

8.9 Pathogenic Role of α-MSH Autoantibodies

Since the initial discovery of α-MSH-reactive autoantibodies in patients with eating disorders in 2002 (Fetissov et al. 2002), several studies have pursued research on their origin, mechanism of action, and clinical relevance. Based on this research, a pathophysiological model of eating disorders has been proposed by Fetissov and Hökfelt in 2019, postulating that both anorexia nervosa and bulimia appear as an autoimmune reaction to the gut bacterial protein ClpB (Fetissov and Hökfelt 2019). Such a model can be contrasted with the pathophysiology of diabetes type 1, where an autoimmune reaction is directed against insulin, a protein hormone critically involved in the regulation of glucose metabolism. In the case of eating disorders, bacterial ClpB is naturally present in healthy gut microbiota and physiologically participates in signaling satiety. An autoimmune reaction against ClpB, leading to production of pathogenic α-MSH-cross reactive IgG, may lead to the chronic activation of the melanocortin system with a loss of appetite and increased anxiety in both anorexia nervosa and bulimia. Neutralization of ClpB may cause a bulimic attack, also explaining why both anorexia and bulimia typically occur in the same patient. If this pathophysiological model is correct, then therapeutic strategies aimed at the elimination of the antigen and/or of the pathogenic α-MSH-autoantibodies may prove effective.

8.10 Conclusions

Neuropeptide-reactive IgG are involved in neuroendocrine signaling as carriers of neuropeptides, protecting them from degradation and as modulators of neuropeptide receptor activation. It is possible that such a role of IgG has evolved as the immune response to the neuropeptide-like microbial antigens constitutively present in the microbiota of all animals with a gut. It is clear that further analysis of autoantibodies that are reactive with neuropeptides/peptide hormones will allow a better understanding of their role in neuroendocrine signaling as well as their involvement in both physiological and pathophysiological regulation of motivated behavior and metabolism.

8.11 Key References

Fetissov, S. O. & Hökfelt, T. 2019. On the origin of eating disorders: altered signaling between gut microbiota, adaptive immunity and the brain melanocortin system regulating feeding behavior. *Curr Opin Pharmacol,* 48, 82–91.

This paper takes you on a 20-year journey, from the finding of α-MSH-reactive antibodies to the formulation of the pathophysiological model of eating disorders.

Takagi, K., Legrand, R., Asakawa, A., Amitani, H., François, M., Tennoune, N., Coëffier, M., Claeyssens, S., Do Rego, J.-C., Déchelotte, P., Inui, A. & Fetissov, S. O. 2013. Anti-ghrelin immunoglobulins modulate ghrelin stability and its orexigenic effect in obese mice and humans. *Nat Commun,* 4:2685.

This paper provides the first evidence of plasma IgG playing the role of a functional peptide hormone carrier, protecting it from degradation.

Værøy, H., Adori, C., Legrand, R., Lucas, N., Breton, J., Cottard, C., Do Rego, J.-C., Duparc, C., Louiset, E., Lefebvre, H., Déchelotte, P., Western, E., Andersson, S., Hökfelt, T. & Fetissov, S. O. 2018. Autoantibodies reactive to adrenocorticotropic hormone can alter cortisol secretion in both aggressive and nonaggressive humans. *Proc Natl Acad Sci USA,* 115, E6576-E6584.

This paper reveals the role of IgG in modulating the activity of the hypothalamo-pituitary adrenal axis in humans, i.e., involvement in individual variability of the stress response.

Acknowledgments Studies of neuropeptide autoantibodies are currently funded by the EC as a part of research projects exploring the role of gut microbiota in autism (H2020 "GEMMA") and in anorexia nervosa (ERAnet, "MIGBAN"), as well as by the PTM program of Inserm, France.

References

Achenbach P, Schlosser M, Williams AJ, Yu L, Mueller PW, Bingley PJ, Bonifacio E (2007) Combined testing of antibody titer and affinity improves insulin autoantibody measurement: Diabetes Antibody Standardization Program. Clin Immunol 122:85–90

Bangale Y, Cavill D, Gordon T, Planque S, Taguchi H, Bhatia G, Nishiyama Y, Arnett F, Paul S (2002) Vasoactive intestinal peptide binding autoantibodies in autoimmune humans and mice. Peptides 23:2251–2257

Bendtzen K, Hansen MB, Ross C, Svenson M (1998) High-avidity autoantibodies to cytokines. Immunol Today 19:209–211

Bien-Ly N, Yu YJ, Bumbaca D, Elstrott J, Boswell CA, Zhang Y, Luk W, Lu Y, Dennis MS, Weimer RM, Chung I, Watts RJ (2014) Transferrin receptor (TfR) trafficking determines brain uptake of TfR antibody affinity variants. J Exp Med 211:233–244

Black JL 3rd, Silber MH, Krahn LE, Fredrickson PA, Pankratz VS, Avula R, Walker DL, Slocumb NL (2005) Analysis of hypocretin (orexin) antibodies in patients with narcolepsy. Sleep 28: 427–431

Bouhajja H, Bougacha-Elleuch N, Lucas N, Legrand R, Marrakchi R, Kaveri SV, Jamoussi K, Ayadi H, Abid M, Mnif-Feki M, Fetissov SO (2018) Affinity kinetics of leptin-reactive immunoglobulins are associated with plasma leptin and markers of obesity and diabetes. Nutr Diabetes 8:32

Coquerel Q, Hamze Sinno M, Boukhettala N, Coëffier M, Terashi M, Bole-Feysot C, Breuillé D, Déchelotte P, Fetissov SO (2012) Intestinal inflammation influences α-MSH reactive autoantibodies: relevance to food intake and body weight. Psychoneuroendocrinology 37:94–106

Costa A, Bini P, Hamze-Sinno M, Moglia A, Franciotta D, Sinforiani E, Ravaglia S, Bole-Feysot C, Hökfelt T, Déchelotte P, Fetissov SO (2011) Galanin and alpha-MSH autoantibodies in cerebrospinal fluid of patients with Alzheimer's disease. J Neuroimmunol 240–241:114–120

Davson H, Segal MB (1996) Physiology of the CSF and blood-brain barriers. CRC Press, Boca Raton

Déchelotte P, Breton J, Trotin-Picolo C, Grube B, Erlenbeck C, Bothe G, Fetissov SO, Lambert G (2021) The probiotic strain H. alvei HA4597® improves weight loss in overweight subjects under moderate hypocaloric diet: a proof-of-concept, multicenter randomized, double-blind placebo-controlled study. Nutrients 13:1902

Deloumeau A, Bayard S, Coquerel Q, Déchelotte P, Bole-Feysot C, Carlander B, Cochen De Cock V, Fetissov SO, Dauvilliers Y (2010) Increased immune complexes of hypocretin autoantibodies in narcolepsy. PLoS One 5:e13320

Demeester S, Keymeulen B, Kaufman L, Van Dalem A, Balti EV, Van De Velde U, Goubert P, Verhaeghen K, Davidson HW, Wenzlau JM, Weets I, Pipeleers DG, Gorus FK (2015) Preexisting insulin autoantibodies predict efficacy of otelixizumab in preserving residual β-cell function in recent-onset type 1 diabetes. Diabetes Care 38:644–651

Dicou E, Nerriere V (1997) Evidence that natural autoantibodies against the nerve growth factor (NGF) may be potential carriers of NGF. J Neuroimmunol 75:200–203

Druce MR, Wren AM, Park AJ, Milton JE, Patterson M, Frost G, Ghatei MA, Small C, Bloom SR (2005) Ghrelin increases food intake in obese as well as lean subjects. Int J Obes Relat Metab Disord 29:1130–1136

Espinoza-Garcia AS, Hunot-Alexander C, Martinez-Moreno AG, Vazquez-Solorzano R, Porchas-Quijada M, Reyes-Castillo Z (2021) IgG antibodies reacting with ghrelin and leptin are correlated with body composition and appetitive traits in young subjects. Appetite 168:105685

Fetissov SO (2011) Neuropeptide autoantibodies assay. Methods Mol Biol 789:295–302

Fetissov SO (2017) Role of the gut microbiota in host appetite control: bacterial growth to animal feeding behaviour. Nat Rev Endocrinol 13:11–25

Fetissov SO (2019) Neuropeptide-like signaling in the microbiota-gut-brain axis. Behav Brain Sci 42:e70

Fetissov SO, Hökfelt T (2019) On the origin of eating disorders: altered signaling between gut microbiota, adaptive immunity and the brain melanocortin system regulating feeding behavior. Curr Opin Pharmacol 48:82–91

Fetissov SO, Hallman J, Oreland L, Af Klinteberg B, Grenbäck E, Hulting AL, Hökfelt T (2002) Autoantibodies against α-MSH, ACTH, and LHRH in anorexia and bulimia nervosa patients. Proc Natl Acad Sci USA 99:17155–17160

Fetissov SO, Harro J, Jaanisk M, Järv A, Podar I, Allik J, Nilsson I, Sakthivel P, Lefvert AK, Hökfelt T (2005) Autoantibodies against neuropeptides are associated with psychological traits in eating disorders. Proc Natl Acad Sci USA 102:14865–14870

Fetissov SO, Hallman J, Nilsson I, Lefvert AK, Oreland L, Hökfelt T (2006) Aggressive behavior linked to corticotropin-reactive autoantibodies. Biol Psychiatry 60:799–802

Fetissov SO, Hamze Sinno M, Coëffier M, Bole-Feysot C, Ducrotté P, Hökfelt T, Déchelotte P (2008) Autoantibodies against appetite-regulating peptide hormones and neuropeptides: putative modulation by gut microflora. Nutrition 24:348–359

François M, Schaefer JM, Bole-Feysot C, Déchelotte P, Verhulst FC, Fetissov SO (2015) Ghrelin-reactive immunoglobulins and anxiety, depression and stress-induced cortisol response in adolescents. The TRAILS study. Prog Neuro-Psychoph 59:1–7

François M, Takagi K, Legrand R, Lucas N, Beutheu Youmba S, Bôle-Feysot C, Cravezic A, Tennoune N, Do Rego J-C, Coëffier M, Inui A, Déchelotte P, Fetissov SO (2016) Increased

ghrelin but low ghrelin-reactive immunoglobulins in a rat model of methotrexate chemotherapy-induced anorexia. Front Nutr 3

Gallas S, Fetissov SO (2011) Ghrelin, appetite and gastric electrical stimulation. Peptides 32:2283–2289

Gallas S, Sinno MH, Boukhettala N, Coëffier M, Dourmap N, Gourcerol G, Ducrotté P, Déchelotte P, Leroi A-M, Fetissov SO (2011) Gastric electrical stimulation increases ghrelin production and inhibits catecholaminergic brainstem neurons in rats. Eur J Neurosci 33:276–284

Garcia FD, Coquerel Q, Kiive E, Déchelotte P, Harro J, Fetissov SO (2011) Autoantibodies reacting with vasopressin and oxytocin in relation to cortisol secretion in mild and moderate depression. Prog Neuro-Psychopharmacol Biol Psychiatry 35:118–125

Garcia FD, Coquerel Q, Do Rego J-C, Cravezic A, Bole-Feysot C, Kiive E, Déchelotte P, Harro J, Fetissov SO (2012) Anti-neuropeptide Y plasma immunoglobulins in relation to mood and appetite in depressive disorder. Psychoneuroendocrinology 37:1457–1467

Hamze Sinno M, Do Rego JC, Coëffier M, Bole-Feysot C, Ducrotte P, Gilbert D, Tron F, Costentin J, Hökfelt T, Déchelotte P, Fetissov SO (2009) Regulation of feeding and anxiety by α-MSH reactive autoantibodies. Psychoneuroendocrinology 34:140–149

Hökfelt T, Barde S, Xu ZD, Kuteeva E, Rüegg J, Le Maitre E, Risling M, Kehr J, Ihnatko R, Theodorsson E, Palkovits M, Deakin W, Bagdy G, Juhasz G, Prud'homme HJ, Mechawar N, Diaz-Heijtz R, Ögren SO (2018) Neuropeptide and small transmitter coexistence: fundamental studies and relevance to mental illness. Front Neural Circuits 12:106

Hortin GL, Sviridov D, Anderson NL (2008) High-abundance polypeptides of the human plasma proteome comprising the top 4 logs of polypeptide abundance. Clin Chem 54:1608–1616

Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, Benveniste H, Vates GE, Deane R, Goldman SA, Nagelhus EA, Nedergaard M (2012) A paravascular pathway facilitates CSF flow through the brain parenchyma and the vlearance of interstitial solutes, including amyloid β. Sci Transl Med 4:147ra111

Karaiskos D, Mavragani CP, Sinno MH, Déchelotte P, Zintzaras E, Skopouli FN, Fetissov SO, Moutsopoulos HM (2010) Psychopathological and personality features in primary Sjogren's syndrome—associations with autoantibodies to neuropeptides. Rheumatology 49:1762–1769

Ketchesin KD, Stinnett GS, Seasholtz AF (2017) Corticotropin-releasing hormone-binding protein and stress: from invertebrates to humans. Stress 20:449–464

Kouhi A, Pachipulusu V, Kapenstein T, Hu P, Epstein AL, Khawli LA (2021) Brain disposition of antibody-based therapeutics: dogma, approaches and perspectives. Int J Mol Sci 22:6442

Lacroix-Desmazes S, Kaveri SV, Mouthon L, Ayouba A, Malanchère E, Coutinho A, Kazatchkine MD (1998) Self-reactive antibodies (natural autoantibodies) in healthy individuals. J Immunol Methods 216:117–137

Lahaye E, Dubessy C, Værøy H, FetissovSO (2021) Oxytocin receptor activation in humans is modulated by oxytocin-reactive immunoglobulin. PNIRS annual meeting, May 20–21st 2021 virtual

Legrand R, Lucas N, Breton J, Azhar S, Do Rego J-C, Déchelotte P, Coëffier M, Fetissov SO (2016) Ghrelin treatment prevents development of activity based anorexia in mice. Eur Neuropsychopharmacol 26:948–958

Legrand R, Lucas N, Dominique M, Azhar S, Deroissart C, Le Solliec M-A, Rondeaux J, Nobis S, Guérin C, Léon F, Do Rego J-C, Pons N, Le Chatelier E, Ehrlich SD, Lambert G, Déchelotte P, Fetissov SO (2020) Commensal Hafnia alvei strain reduces food intake and fat mass in obese mice-a new potential probiotic for appetite and body weight management. Int J Obes 44:1041–1051

Leng G, Ludwig M (2008) Neurotransmitters and peptides: whispered secrets and public announcements. J Physiol 586:5625–5632

Lucas N, Legrand R, Bôle-Feysot C, Breton J, Coëffier M, Akkermann K, Järv A, Harro J, Déchelotte P, Fetissov SO (2019) Immunoglobulin G modulation of the melanocortin 4 receptor signaling in obesity and eating disorders. Transl Psychiatry 9:87

Mahoney CE, Cogswell A, Koralnik IJ, Scammell TE (2019) The neurobiological basis of narcolepsy. Nat Rev Neurosci 20:83–93

Molfino A, Amabile MI, Imbimbo G, Emiliani A, Ramaccini C, Lahaye E, Takagi K, Fetissov SO (2020) Plasma enterobacterial ClpB levels and ClpB- and α-MSH-reactive immunoglobulins in lung cancer patients with and without anorexia. Nutrition 78:110952

Myagkova MA, Gavrilova SI, Lermontova NN, Kalyn YB, Selezneva ND, Zharikov GA, Kolykhalov IV, Abramenko TV, Serkova TP, Bachurin SO (2003) Content of autoantibodies to bradykinin and beta-amyloid(1-42) as a criterion for biochemical differences between Alzheimer's dementias. Bull Exp Biol Med 136:49–52

Nevinsky GA, Buneva VN (2002) Human catalytic RNA- and DNA-hydrolyzing antibodies. J Immunol Methods 269:235–249

Nishii N, Takasu M, Kojima M, Hachisu T, Wakabayashi K, Iwasawa A, Maeda S, Ohba Y, Kitagawa H (2010) Presence of anti-insulin natural autoantibodies in healthy cats and its interference with immunoassay for serum insulin concentrations. Domest Anim Endocrinol 38:138–145

Paul S, Said SI (1988) Human autoantibody to vasoactive intestinal peptide: increased incidence in muscular exercise. Life Sci 43:1079–1084

Paul S, Heinz-Erian P, Said SI (1985) Autoantibody to vasoactive intestinal peptide in human circulation. Biochem Biophys Res Commun 130:479–485

Paul S, Said SI, Thompson AB, Volle DJ, Agrawal DK, Foda H, De La Rocha S (1989) Characterization of autoantibodies to vasoactive intestinal peptide in asthma. J Neuroimmunol 23:133–142

Paul S, Gao QS, Huang H, Sun M, Thompson A, Rennard S, Landers D (1995) Catalytic autoantibodies to vasoactive intestinal peptide. Chest 107:125S–126S

Porchas-Quijada M, Reyes-Castillo Z, Muñoz-Valle JF, Durán-Barragán S, Aguilera-Cervantes V, López-Espinoza A, Vázquez-Del Mercado M, Navarro-Meza M, López-Uriarte P (2019) IgG anti-ghrelin immune complexes are increased in rheumatoid arthritis patients under biologic therapy and are related to clinical and metabolic markers. Front Endocrinol (Lausanne) 10:252

Roopenian DC, Christianson GJ, Sproule TJ, Brown AC, Akilesh S, Jung N, Petkova S, Avanessian L, Choi EY, Shaffer DJ, Eden PA, Anderson CL (2003) The MHC class I-like IgG receptor controls perinatal IgG transport, IgG homeostasis, and fate of IgG-Fc-coupled drugs. J Immunol 170:3528–3533

Roubalova R, Prochazkova P, Dvorak J, Hill M, Papezova H, Kreisinger J, Bulant J, Lambertova A, Holanova P, Bilej M, Tlaskalova-Hogenova H (2021) Altered serum immunological and biochemical parameters and microbiota composition in patients with AN during realimentation. Front Nutr 8:680870

Schaefer JM, Fetissov SO, Legrand R, Claeyssens S, Hoekstra PJ, Verhulst FC, Van Oort FVA (2013) Corticotropin (ACTH)-reactive immunoglobulins in adolescents in relation to antisocial behavior and stress-induced cortisol response. The TRAILS study. Psychoneuroendocrinology 38:3039–3047

Schlachetzki F, Zhu C, Pardridge WM (2002) Expression of the neonatal Fc receptor (FcRn) at the blood–brain barrier. J Neurochem 81:203–206

Sebriakova M, Little JA (1973) A method for the determination of plasma insulin antibodies and its application in normal and diabetic subjects. Diabetes 22:30–40

Sodoyez JC, Sodoyez-Goffaux F, Koch M, Sondag D, Bouillenne C, François-Gérard C, Bosi E (1990) Clonally restricted insulin autoantibodies in a cohort of 2200 healthy blood donors. Diabetologia 33:719–725

St-Amour I, Paré I, Alata W, Coulombe K, Ringuette-Goulet C, Drouin-Ouellet J, Vandal M, Soulet D, Bazin R, Calon F (2013) Brain bioavailability of human intravenous immunoglobulin and its transport through the murine blood-brain barrier. J Cereb Blood Flow Metab 33:1983–1992

Stawerska R, Czkwianianc E, Matusiak A, Smyczyńska J, Hilczer M, Chmiela M, Lewiński A (2015) Prevalence of autoantibodies against some selected growth and appetite-regulating

neuropeptides in serum of short children exposed to Candida albicans colonization and/or Helicobacter pylori infection: the molecular mimicry phenomenon. Neuro Endocrinol Lett 36: 458–464

Surina-Baumgartner DM, Langhans W, Geary N (1995) Hepatic portal insulin antibody infusion increases, but insulin does not alter, spontaneous meal size in rats. Am J Phys 269:R978–R982

Takagi K, Legrand R, Asakawa A, Amitani H, François M, Tennoune N, Coëffier M, Claeyssens S, Do Rego J-C, Déchelotte P, Inui A, Fetissov SO (2013) Anti-ghrelin immunoglobulins modulate ghrelin stability and its orexigenic effect in obese mice and humans. Nat Commun 4:2685

Tanaka S, Honda Y, Inoue Y, Honda M (2006) Detection of autoantibodies against hypocretin, hcrtrl, and hcrtr2 in narcolepsy: anti-Hcrt system antibody in narcolepsy. Sleep 29:633–638

Tennoune N, Chan P, Breton J, Legrand R, Chabane YN, Akkermann K, Jarv A, Ouelaa W, Takagi K, Ghouzali I, François M, Lucas N, Bole-Feysot C, Pestel-Caron M, Do Rego JC, Vaudry D, Harro J, Dé E, Déchelotte P, Fetissov SO (2014) Bacterial ClpB heat-shock protein, an antigen-mimetic of the anorexigenic peptide [alpha]-MSH, at the origin of eating disorders. Transl Psychiatry 4:e458

Tennoune N, Legrand R, Ouelaa W, Breton J, Lucas N, Bole-Feysot C, Rego J-CD, Déchelotte P, Fetissov SO (2015) Sex-related effects of nutritional supplementation of Escherichia coli: Relevance to eating disorders. Nutrition 31:498–507

Terashi M, Asakawa A, Harada T, Ushikai M, Coquerel Q, Sinno MH, Déchelotte P, Inui A, Fetissov SO (2011) Ghrelin reactive autoantibodies in restrictive anorexia nervosa. Nutrition 27: 407–413

Værøy H, Adori C, Legrand R, Lucas N, Breton J, Cottard C, Do Rego J-C, Duparc C, Louiset E, Lefebvre H, Déchelotte P, Western E, Andersson S, Hökfelt T, Fetissov SO (2018) Autoantibodies reactive to adrenocorticotropic hormone can alter cortisol secretion in both aggressive and nonaggressive humans. Proc Natl Acad Sci USA 115:E6576–E6584

Vaeroy H, Schneider F, Fetissov SO (2019) Neurobiology of aggressive behavior—role of autoantibodies reactive with stress-related peptide hormones. Front Psychiatry 10

Wallenius M, Lind A, Akel O, Karlsson E, Svensson M, Arvidsson E, Ramelius A, Törn C, Palm L, Lernmark Å, Elding Larsson H (2019) Autoantibodies in Pandemrix®-induced narcolepsy: nine candidate autoantigens fail the conformational autoantibody test. Autoimmunity 52:185–191

Wan X, Thomas JW, Unanue ER (2016) Class-switched anti-insulin antibodies originate from unconventional antigen presentation in multiple lymphoid sites. J Exp Med 213:967–978

Wang W, Wang EQ, Balthasar JP (2008) Monoclonal antibody pharmacokinetics and pharmacodynamics. Clin Pharmacol Ther 84:548–558

Yu J, Gimenez LE, Hernandez CC, Wu Y, Wein AH, Han GW, Mcclary K, Mittal SR, Burdsall K, Stauch B, Wu L, Stevens SN, Peisley A, Williams SY, Chen V, Millhauser GL, Zhao S, Cone RD, Stevens RC (2020) Determination of the melanocortin-4 receptor structure identifies Ca2+ as a cofactor for ligand binding. Science 368:428–433

Zlokovic BV, Skundric DS, Segal MB, Lipovac MN, Mackic JB, Davson H (1990) A saturable mechanism for transport of immunoglobulin G across the blood-brain barrier of the guinea pig. Exp Neurol 107:263–270