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How to contribute to the progress of neuroendocrinology: New insights from discovering novel neuropeptides and neurosteroids regulating pituitary and brain functions

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ABSTRACT

Obtaining new insights by discovering novel neuropeptides and neurosteroids regulating pituitary and brain functions is essential for the progress of neuroendocrinology. At the beginning of 1970s, gonadotropin-releasing hormone (GnRH) was discovered in mammals. Since then, it was generally accepted that GnRH is the only hypothalamic neuropeptide regulating gonadotropin release in vertebrates. In 2000, however, gonadotropin-inhibitory hormone (GnIH), a novel hypothalamic neuropeptide that actively inhibits gonadotropin release, was discovered in quail. The follow-up studies demonstrated that GnIH acts as a new key player for regulation of reproduction across vertebrates. It now appears that GnIH acts on the pituitary and the brain to serve a number of behavioral and physiological functions. On the other hand, a new concept has been established that the brain synthesizes steroids, called neurosteroids. The formation of neurosteroids in the brain was originally demonstrated in mammals and subsequently in other vertebrates. Recently, 7α-hydroxypregnenolone was discovered as a novel bioactive neurosteroid inducing locomotor behavior of vertebrates, indicating that neurosteroidogenesis in the brain is still incompletely elucidated in vertebrates. At the beginning of 2010s, it was further found that the pineal gland actively produces neurosteroids. Pineal neurosteroids act on the brain to regulate locomotor rhythms and neuronal survival. Furthermore, the interaction of neuropeptides and neurosteroids is becoming clear. GnIH decreases aggressive behavior by regulating neuroestrogen synthesis in the brain. This review summarizes these new insights by discovering novel neuropeptides and neurosteroids in the field of neuroendocrinology.

1. Overview

In the 1920s, the new concept of "neurosecretion" was first proposed by Ernst and Berta Scharrer, who considered that hypothalamic neurons, which terminate at the neurohypophysis, secrete hormones produced in these neurons as neurohormones. This seminal idea was not accepted easily by the scientific society in those days. However, the concept of "neurosecretion" proposed by Ernst and Berta Scharrer was established by Bargmann in 1949. The discovery of neurosecretion in the first half of the 20th century led to the creation of neuroendocrinology, a new research field of endocrinology. Subsequently, oxytocin (Livermore and Du Vigneaud, 1949) and vasopressin (Turner et al., 1951), hypothalamic neuropeptides, were identified to be neurohormones that are secreted from the neurohypophysis.

On the other hand, based on the morphology of hypothalamic neurons that terminate at the median eminence (ME), Harris (1948) hypothesized that these hypothalamic neurons may secrete neurohormones from the ME into the hypophysial portal system to regulate the secretion of anterior pituitary hormones. Subsequently, the groups of Schally and Guillemin supported this hypothesis by the discoveries of various important neurohormones, such as thyrotropin-releasing hormone (TRH) (Burgus et al., 1969; Boler et al., 1969), gonadotropin-releasing hormone (GnRH) (Matsuo et al., 1971; Burgus et al., 1972) and growth hormone-inhibiting hormone (somatostatin) (Brazeau et al., 1973), in the brain of mammals.

At the beginning of the 1970s, GnRH, a hypothalamic neuropeptide known to stimulate the release of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from gonadotropes in the anterior pituitary, was discovered in mammals by Schally

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(Matsuo et al., 1971) and Guillemin (Burgus et al., 1972) who were awarded a Nobel Prize in 1977 for the discoveries of GnRH and other hypothalamic neuropeptides. Since the discovery of GnRH in mammals, several GnRHs have been identified in other vertebrates (King and Millar, 1982; Miyamoto et al., 1982, 1984; Sherwood et al., 1986). Based on tremendous amounts of data, until recently, it was generally believed that GnRH is the only hypothalamic neuropeptide regulating gonadotropin release, and that GnRH neurons serve as the final pathway through which the brain regulates reproduction in vertebrates.

How can we contribute to the progress of neuroendocrinology? Discoveries of novel neuropeptides regulating pituitary and brain functions are essential to obtain new insights in the field of neuroendocrinology. In 2000, we provided a new concept on the basis of the discovery of gonadotropin-inhibitory hormone (GnIH), a hypothalamic neuropeptide that actively reduces gonadotropin release, in quail (Tsutsui et al., 2000a). The follow-up studies demonstrated that GnIH is highly conserved among vertebrates, from agnathans to humans, and that it acts as a key player for regulation of reproduction (for reviews, see Kriegsfeld et al., 2015; Tsutsui, 2009; Tsutsui and Ubuka, 2012; Tsutsui and Ukena, 2006; Tsutsui et al., 2006b, 2007, 2010a,b, 2012b, 2013d; Ukena and Tsutsui, 2005). In addition, recent studies have demonstrated that GnIH has other important functions beyond reproduction (Tobari et al., 2014; Ubuka et al., 2014). It now appears that GnIH acts on the pituitary and the brain to serve a number of behavioral and physiological functions other than regulation of reproduction. Thus, following 14 years of GnIH research in collaboration with the laboratories of Wingfield, Bentley, Kriegsfeld, Clarke, Sower, and others has obtained new insights in the field of neuroendocrinology (for reviews, see Kriegsfeld et al., 2015; Tsutsui, 2009; Tsutsui and Ubuka, 2012; Tsutsui et al., 2006b, 2007, 2010a,b, 2012b, 2013d; Ubuka et al., 2013).

As mentioned above, the identification of novel neuropeptides greatly contributed to the progress of neuroendocrinology. On the other hand, for a long time, the brain has been considered as a target site for steroid hormones secreted by peripheral endocrine organs, such as the adrenal gland, gonads and placenta (only in mammals). However, a new concept has been established that the brain itself is also the site synthesizing steroids *de novo* from cholesterol (CHOL). These brain-born steroids, called neurosteroids, have been found to exert a variety of biological actions, such as proliferation, differentiation, activity and survival of nerve cells, and control of behavioral, neuroendocrine and metabolic processes (for reviews, see Baulieu, 1997; Compagnone and Mellon, 2000; Do-Rego et al., 2009; Mellon and Vaudry, 2001; Tsutsui and Mellon, 2006; Tsutsui et al., 1999, 2000b, 2003, 2006a). The formation of neurosteroids in the brain was originally demonstrated in mammals by the group of Baulieu 30 years ago (Compagnone et al., 1995; Corpéchot et al., 1981, 1983; Jo et al., 1989; Lanthier and Patwardhan, 1986; Mathur et al., 1993; Mellon and Deschepper, 1993; Robel and Baulieu, 1985; Robel et al., 1987). *De novo* synthesis and biological actions of neurosteroids in the brain of other vertebrates have also been established by my group and the groups of Vaudry, Schlinger, Kah, and others (in birds: Freking et al., 2000; London and Schlinger, 2007; London et al., 2003, 2006, 2010; Matsunaga et al., 2001, 2002; Schlinger et al., 1999; Soma et al., 2004; Tam and Schlinger, 2007; Tsutsui and Schlinger, 2001; Tsutsui and Yamazaki, 1995; Tsutsui et al., 1997, 1999, 2003; Ukena et al., 1999, 2001; Usui et al., 1995; Vanson et al., 1996; in amphibians: Beaujean et al., 1999; Bruzzone et al., 2010; Do-Rego et al., 2007; Inai et al., 2003; Matsunaga et al., 2004b; Mensah-Nyagan et al., 1994, 1996a, 1996b, 1999; Takase et al., 1999, 2002, 2011; in fish: Brion et al., 2012; Diotel et al., 2011; Menuet et al., 2005; Sakamoto et al., 2001).

Thus, neurosteroidogenesis in the brain is a conserved property across vertebrates. However, the biosynthetic pathways leading to the formation of neurosteroids in the brain have only incompletely been elucidated in vertebrates (for a review, Tsutsui et al., 2006a). In fact, at the beginning of 2000s, 7α-hydroxypregnenolone (7α-OH PREG) was detected as a novel bioactive neurosteroid inducing locomotor behavior, in the brain of newts and quail (Haraguchi et al., 2010; Matsunaga et al., 2004b; Tsutsui et al., 2008). Over the past decade, 7α-OH PREG appeared to play a key role in the regulation of locomotor behavior via the dopaminergic system across vertebrates, providing a new insight in the field of neurosteroid research (for reviews, see Tsutsui et al., 2009a,b, 2010c,d, 2012a, 2013a–c).

Until recently, it was generally believed that neurosteroids are produced in neurons and glial cells in the brain and in other parts of the central and peripheral nervous systems (for reviews, see Baulieu, 1997; Compagnone and Mellon, 2000; Do-Rego et al., 2009; Mellon and Vaudry, 2001; Tsutsui and Mellon, 2006; Tsutsui et al., 1999, 2000b, 2003, 2006a). At the beginning of 2010s, however, it was found that the pineal gland, an endocrine organ located close to the brain, is an important site of *de novo* production of neurosteroids from CHOL (Haraguchi et al., 2012a; Hatori et al., 2011). This is a paradigm shift of neurosteroid formation. Furthermore, the pineal gland secretes actively 7α-OH PREG and 3α,5α-tetrahydroprogesterone (3α,5α-THP) as major pineal neurosteroids that are involved in locomotor rhythms and neuronal survival, respectively (Haraguchi et al., 2012a; Hatori et al., 2011). Thus, the discovery of pineal neurosteroids has also obtained a new insight in the field of neurosteroid research.

This review summarizes new insights into the neuroendocrinology of vertebrates, based on our studies over the past decade of novel neuropeptides and neurosteroids regulating pituitary and brain functions. In addition, new findings obtained by recent studies focused on the interaction of neuropeptides and neurosteroids are highlighted. For detailed information the reader is referred to other reviews and minireviews regarding the structures, biosyntheses and biological actions of GnIH and brain and pineal neurosteroids in this special issue.

2. A new insight in neuroendocrinology by discovering GnIH, a novel hypothalamic neuropeptide involved in reproduction and reproductive behavior

2.1. Background and overview

Since the discovery of GnRH in mammals by Schally (Matsuo et al., 1971) and Guillemin (Burgus et al., 1972), it was generally accepted that GnRH is the only hypothalamic neuropeptide regulating pituitary gonadotropin release, and that GnRH neurons serve as the final pathway through which the brain regulates reproduction in vertebrates. However, the recent discoveries of novel hypothalamic neuropeptides that regulate reproduction have broadened the horizon of neuroendocrinology. One of such important discoveries was that of GnIH in the avian brain (Tsutsui et al., 2000a). This review highlights the discovery of GnIH and the advances made in our understanding of GnIH structures and biological actions in birds and other vertebrates as a new insight in neuroendocrinology.

2.2. How to identify GnIH in the brain

GnIH was first discovered in the brain of the Japanese quail while searching a novel RFamide peptide, which has a C-terminal Arg-Phe-NH_2 motif (Tsutsui et al., 2000a). RFamide peptides were originally isolated from invertebrates in the late 1970s. The first