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## **Time is of the essence - investigating kinetic interactions between drug, endogenous neuropeptides and receptor**

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# Chapter 2

## Kinetic profile of neuropeptide – receptor interactions

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## **Abstract**

Currently, drug discovery focusses only on quantifying pharmacological parameters, sometimes including binding kinetics, of drug candidates. For a complete understanding of a drug's desired binding kinetics, the kinetics of both the target and its endogenous ligands should be considered. This is because the release and binding kinetics of endogenous ligands in addition to receptor internalization rates are significant contributors to drug-target interactions.

Here, we discuss the kinetic profile of three neuropeptides and their receptors; gonadotropin-releasing hormone receptor (GnRHR), neuropeptide Y receptors, and corticotropin-releasing factor receptor 1 (CRF<sub>1</sub>R). These three examples provide new insights into the importance of kinetic profiles which could improve the understanding of desired drug-target binding kinetics and advance drug discovery for various neurological and psychiatric illnesses.

## Background of neuropeptides in drug discovery

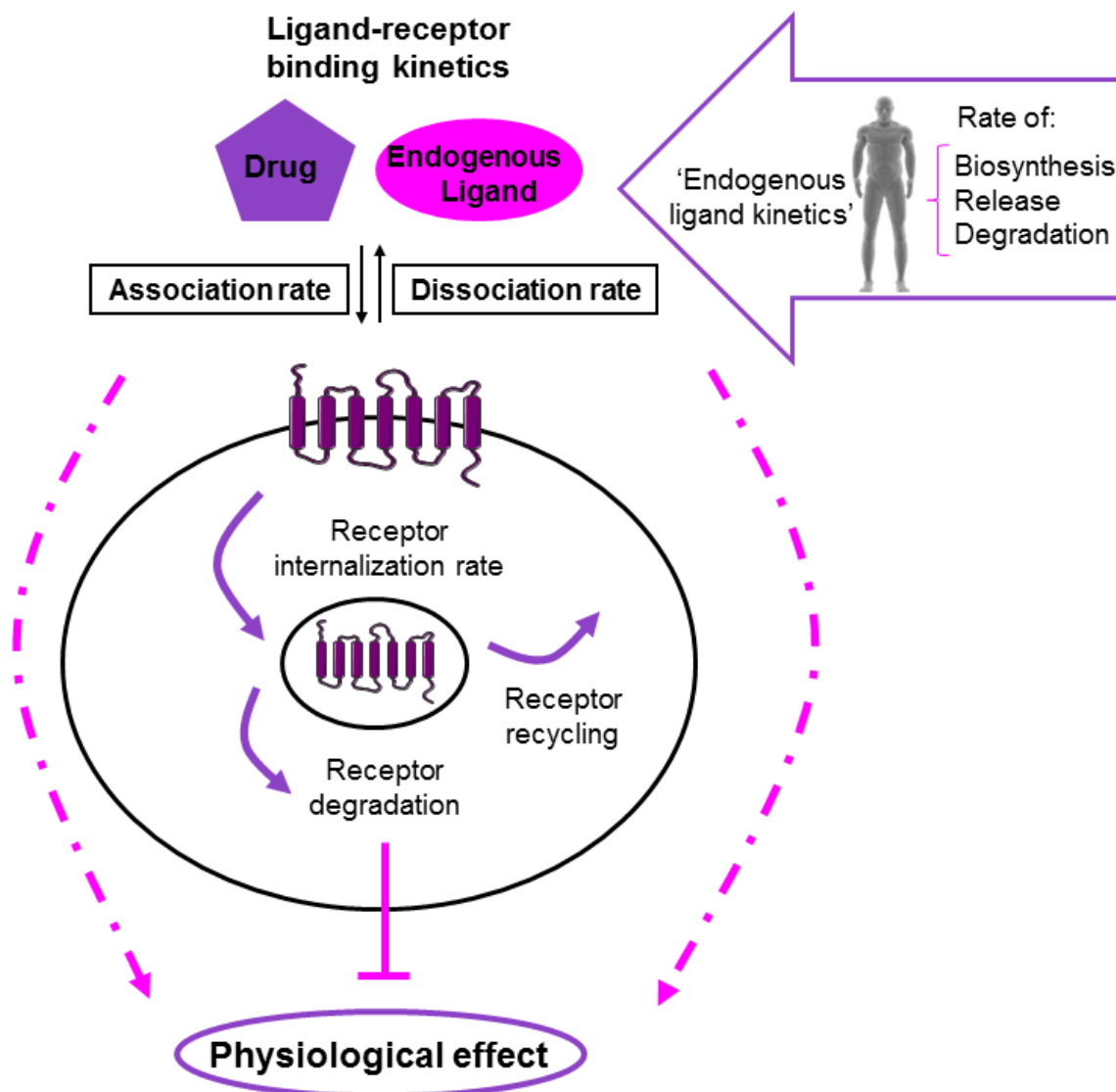
Over the past 40 years, many neuropeptides have been identified in the central nervous system (CNS) and the peripheral nervous system (PNS). Neuropeptides are 3-100 amino acid long polypeptides and are synthesized by neurons. Neuropeptides act on either neural substrates, such as neurons and glial cells or on non-neuronal target cells [1]; they mediate neuronal communication by acting on neuropeptide receptors. Neuropeptide receptors include over 44 receptor families, of which most are G protein-coupled receptors (GPCRs). Neuropeptides and their cognate receptors are involved in many physiological and behavioral functions, such as pain regulation, blood pressure, body temperature, feeding behavior, reproduction, sleep, and learning and memory [2]. Therefore, neuropeptide transmission is an attractive area for drug discovery in several therapeutic areas, including inflammatory conditions [3], epilepsy [4] and psychiatric diseases [5]. Release of endogenous neuropeptides is often pulsatile or in bursts in response to stress, resulting in instant high local concentrations which adds complexity to the development of drugs targeting neuropeptide receptors [6].

## Optimized ligand-receptor binding kinetics is an emerging concept in drug discovery research

Many drug candidates have failed in clinical trials, over 50% due to a reported lack of efficacy [7]. Several studies suggest that binding kinetics, particularly the lifetime of the ligand-receptor binary complex, may be more relevant for *in vivo* drug efficacy than their typical equilibrium parameters obtained *in vitro*, such as target affinity ( $K_i$ ) and potency ( $IC_{50}$ ) [8-10]. This lifetime can be expressed as the drug-target residence time (RT) and is reflected by the dissociation rate constant ( $k_{off}$ ) of the ligand or drug. The  $k_{off}$  value can simply be converted to RT, which is equal to the reciprocal of  $k_{off}$  ( $RT = 1/k_{off}$ ).

Currently, several successfully marketed drugs in the GPCR field have been proven in retrospect to have long RT [11]. These drugs illustrate the benefits of optimized binding kinetics in drug discovery represented by lower dosages, increased efficacy and/or safety. For example, the NK1 receptor antagonist aprepitant has superior *in vivo* efficacy in comparison to other NK1 receptor antagonists due to its slow receptor dissociation [11]. As another example, patients with asthma or chronic obstructive pulmonary disease (COPD) can benefit from the slowly dissociating  $\beta_2$ -adrenoreceptor agonist olodaterol [12]. The bronchodilating effects of this drug last up to 24 h. which allows for once-daily administration. However, it is important to note that slow dissociation rates are not always desired. Long RT can also lead to adverse effects and thereby decrease drug safety in the patient [10]. An example of a successful drug with a short residence time is quetiapine, a dopamine  $D_2$

receptor antagonist approved for the treatment of schizophrenia and bipolar disorder. This antipsychotic drug was shown to have fewer (on-target) side effects than other dopamine D<sub>2</sub> receptor antagonists on the market [13]. Altogether, incorporating optimized binding kinetics prospectively could improve the success rate in drug discovery and development and thus of drugs entering the market.

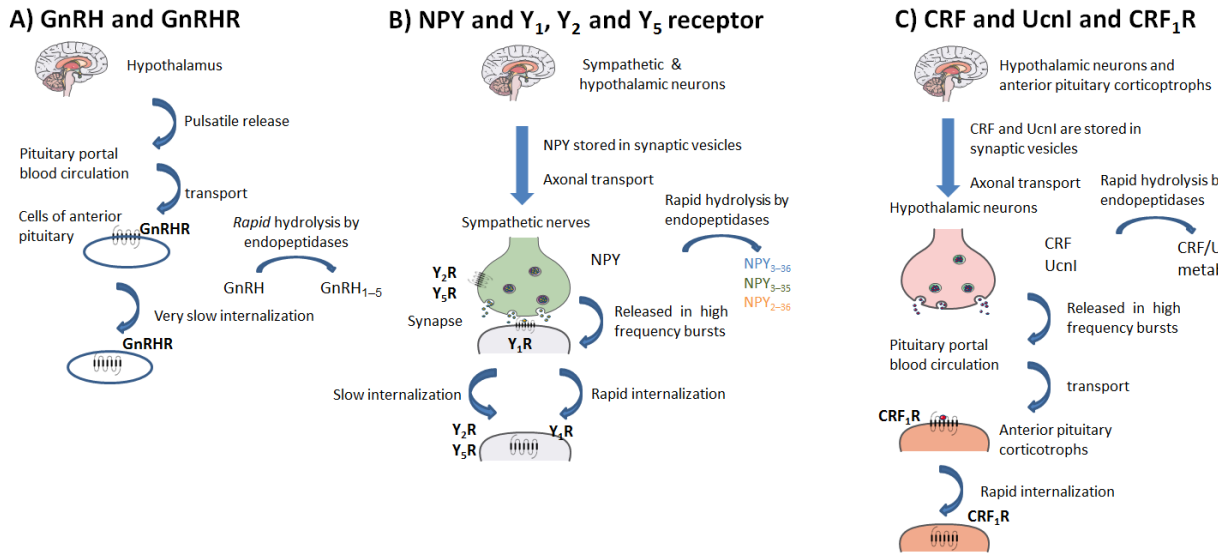


**Figure 1.** Schematic representation of the structure of this review. Drugs are often competing with endogenous ligands but the kinetic profile of the target receptor and its endogenous ligand(s) are often overlooked. In this review the endogenous ligand release kinetics, endogenous ligand binding kinetics and receptor fate (e.g., internalization kinetics and degradation pathways), i.e. kinetic profiles, of three exemplary and diverse neuropeptide receptor classes and their endogenous ligands will be discussed.

## The kinetic profile of a target receptor and its endogenous ligand

The majority of successful drugs achieve their effect by competing with endogenous ligands for the same binding site. Therefore, understanding the pharmacological and physiological behavior of endogenous ligands in the human body is crucial. In contrast to the *in vitro* test tube situation, the human body is an open system. Consequently, the concentration of endogenous ligand, drug and target receptor change over time as these molecules enter and leave the system [10, 14]. Moreover, in order to comprehend desired drug-target kinetics, awareness of the kinetic profile of the target receptor and its endogenous ligand(s) is essential. Firstly, it is imperative to consider the time scale and rate of endogenous ligand release as this can result in temporarily high local concentrations. Secondly, knowledge of the rates of association to and dissociation from the receptor not only of the drug candidate but also of the endogenous ligand should be considered as these parameters can be a limitation to the availability of the unoccupied receptor. Finally, to get a better understanding of the *in vivo* effects of a drug candidate, insight into the rate at which receptors desensitize or internalize under normal and pathophysiological conditions is necessary [15]. Agonist responses are usually regulated by receptor desensitization and internalization and this can limit the effect and duration of receptor signaling [16]. Moreover, receptor complexation with receptor activity-modifying proteins (RAMPs) [17], as well as receptor ubiquitination and other degradation steps are of influence on receptor half-life [18] and although literature on this topic is sparse more knowledge could aid drug discovery [19]. Accordingly, the impact of a long RT drug may be diminished when receptors are rapidly degraded or recycled [15, 20]. Attempts to simultaneously address these aspects in mathematical models that allow such an *in vitro/in vivo* translation are encouraging. These models can be of great value to analyze experimental data and simulate various cases of drug treatment in a comprehensive and integrative fashion [21].

In brief, to improve drug discovery more insight towards the kinetic characteristics of both drug and the endogenous ligand and its target, i.e. the full kinetic profile, is crucial (Figure 1). We propose a new perspective to drug discovery, where increased attention is paid to 1) release frequency of endogenous ligands (Box 1, Box 2 and Figure 2), 2) binding kinetics of endogenous ligands, and 3) internalization and degradation rates of target receptors. To demonstrate the diversity in kinetic profiles of neuropeptide receptors, we provide a synthesis of the kinetic profiles of three exemplary and diverse neuropeptide receptors and their endogenous ligands, i.e. gonadotropin-releasing hormone receptor (GnRHR), neuropeptide Y receptors, and corticotropin-releasing factor receptor 1 (CRF<sub>1</sub>R) (Figure 1).



**Figure 2.** Schematic representation of biosynthesis, release and degradation kinetics ('endogenous ligand kinetics') of endogenous neuropeptides GnRH (A), NPY (B) and CRF (C) in the human body at physiological conditions. This cartoon describes the location of synthesis of the neuropeptide, followed by release, transport, and binding at its cognate receptor. Finally, the endogenous neuropeptide is degraded by endopeptidases. Sources of medical illustrations: Somersault1824 Library of Science & Medical Illustrations [22] and Servier medical art. [23].

## Kinetic profile of neuropeptide receptors and their endogenous ligands

### *GnRH and the GnRHR*

Gonadotropin-releasing hormone (GnRH) is a neuropeptide that mediates the central control of the reproductive system and is released by the hypothalamus. GnRH activates GnRH receptors (GnRHRs) in the anterior pituitary and subsequently stimulates secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). GnRH is released in high and low frequency pulses dependent on gender and reproductive cycles (Box 1 and Figure 2) [24], and plasma concentrations range from 0.1-2.0 pg/ml (i.e. 84.5 nM – 1.7 μM) [25]. GnRHR belongs to the class A rhodopsin-like family of GPCRs and GnRHR is predominantly coupled to Gα<sub>q/11</sub> proteins. A unique feature of the GnRHR is that it, unlike all other GPCRs, lacks an intracellular C-terminal tail [26]. GnRHR is successfully targeted to treat hormone-dependent diseases such as prostate cancer [27] with either antagonists or agonists that act as functional antagonist.

In 1979 a study demonstrated that radiolabeled GnRH (i.e., <sup>125</sup>I-GnRH) associated rapidly to ovine anterior pituitary homogenates with a k<sub>on</sub> value of 0.78 nM<sup>-1</sup> min<sup>-1</sup>.

Dissociation of the agonist was extremely rapid, with a  $k_{\text{off}}$  value of  $0.18 \text{ min}^{-1}$ , translating into a RT of 5.6 min which was calculated from the initial slope of the dissociation curve [28].

More recently [29], two novel competition association assays were developed that allowed for the first time the determination of kinetic receptor binding characteristics of a series of peptide agonists for the human GnRH receptor, including its endogenous ligand GnRH. Firstly, a novel radioligand binding competition association assay was developed in

#### **Box 1: Biosynthesis, release and degradation of endogenous neuropeptides**

Neuropeptides are generally synthesized from larger precursors in the neuronal cell body upon stress stimuli [6]. The precursors are stored in vesicles, where they are degraded by convertases into active peptides. Neuropeptides are transported to the release sites at neurons and released by exocytosis, where they bind their cognate receptor [30]. The kinetics of neuropeptide synthesis, release and degradation is presented in Figure 2.

##### *Gonadotropin-releasing hormone*

- GnRH is synthesized in the hypothalamus from a precursor polypeptide by enzymatic processing [31-33].
- GnRH is released in pulses from the hypothalamus. GnRH secretion is regulated both by the feedback actions of gonadal steroids and neural input from higher cognitive and sensory centers [33]. The pattern of pulsatile GnRH secretion ranges from minutes to hours and varies between sexes, during reproductive life and during the menstrual cycle in females [24] and ranges in frequency between 30 min and 3-4 hours [34].
- GnRH is rapidly hydrolyzed (half-life 2-4 min) into GnRH 1-5 by thimet oligopeptidase (EC 3.4.24.15) both in the hypothalamus and the anterior pituitary [35-37].

##### *Neuropeptide Y*

- NPY is synthesized in the hypothalamus and in the peripheral nervous system by sympathetic neurons [38]
- NPY is released in high frequency bursts (every 5 min) from sympathetic nerve terminals, upon stress stimuli or pathological conditions [39, 40].
- NPY is rapidly hydrolysed (half-life approximately 12 min) by peptidases, including dipeptidyl peptidase IV (EC 3.4.14.5) and aminopeptidase P (EC 3.4.11.9) [41, 42].

##### *Corticotropin-releasing factor and Urocortin I*

- CRF and UcnI are synthesized and released by the paraventricular nucleus of the hypothalamus [43].
- The axons of hypothalamic neurons release CRF and UcnI (approximately every 5 min) into the hypophyseal portal blood in reaction to stress [6].
- CRF and UcnI are rapidly hydrolyzed (half-life 12-73 min) by endothelin-converting enzyme 1 (ECE1, EC 3.4.24.71) in the brain [44, 45].

which GnRH had  $k_{on}$ ,  $k_{off}$  and RT values of  $0.06 \pm 0.01 \text{ nM}^{-1} \text{ min}^{-1}$ ,  $0.2 \pm 0.02 \text{ min}^{-1}$ , and  $6.3 \pm 0.6 \text{ min}$  at room temperature, respectively (Table 1).

Secondly, a homogenous time-resolved fluorescence (TR-FRET) Tag-lite™ method was developed as an alternative assay for the same purpose. These TR-FRET experiments provided similar  $k_{on}$ ,  $k_{off}$  and RT values for GnRH of  $0.02 \pm 0.01 \text{ nM}^{-1} \text{ min}^{-1}$ ,  $0.44 \pm 0.3 \text{ min}^{-1}$ , and  $2.3 \pm 1.6 \text{ min}$  at room temperature, respectively [29] (Table 1).

**Table 1.** Qualitative overview of the kinetic profile, i.e. the release kinetics of the endogenous ligand(s), binding kinetics and receptor internalization rates, of the GnRH receptor, NPY receptors and CRF1 receptor (see also Box 1).

Neuropeptide system		Fast*	Medium*	Slow*
GnRH	Release kinetics**	GnRH	X	X
	Binding kinetics	GnRH-GnRHR	X	
	Internalization kinetics	GnRHR		X
NPY	Release kinetics***	NPY	X	
	Binding kinetics	NPY-Y <sub>1</sub> R	$k_{on}$	$k_{off}$
		NPY-Y <sub>2</sub> R	$k_{on}$	$k_{off}$
		NPY-Y <sub>5</sub> R	$k_{on}$	$k_{off}$
	Internalization kinetics	Y <sub>1</sub> R	X	
		Y <sub>2</sub> R		X
Y <sub>5</sub> R			X	
CRF	Release kinetics***	CRF	X	
	Binding kinetics	UCNI	X	X
		CRF-CRF <sub>1</sub> R	$k_{on}$	$k_{off}$
		UCNI-CRF <sub>1</sub> R		X
	Internalization kinetics	CRF <sub>1</sub> R	males	females

\* Fast, medium and slow kinetics are an arbitrary categorization in proportion to the target system, exact rates can be found in the corresponding paragraph.

\*\* GnRH release is pulsatile ranging from minutes to hours and depends on gender, age and menstrual cycle, see also Box 1.

\*\*\* NPY, CRF and UCNI are released in high frequency bursts in response to stress or pathological conditions, see also Box 1.

The lack of an intracellular C-terminal tail on the GnRH receptor results in the absence of rapid arrestin-mediated desensitization and in very slow internalization rates [26, 46]. Madziva *et al.* showed less than 50% internalization after 60 min stimulation with a GnRH analogue [47]. Additionally, Pawson *et al.* showed that mammalian GnRHRs (human

and rat) undergo slow, constitutive (i.e. agonist-independent) internalization [46]. The importance of a C-terminal tail for receptor internalization was shown by two studies; firstly, the catfish GnRH receptor that does possess an intracellular C-terminal tail displayed rapid desensitization and internalization. It was shown that approximately 50% of the catfish GnRH receptors were internalized after 15 min stimulation with chicken II GnRH (endocytosis rate constant =  $0.099 \text{ min}^{-1}$ ) [48]. Secondly, addition of a functional intracellular C-terminal tail of the thyrotropin-releasing hormone receptor (TRHR) to the rat GnRHR produced rapid desensitization and increased receptor internalization rates [48].

In brief, drugs targeting the GnRHR are competing with fast association and dissociation kinetics of endogenous GnRH that is released in pulses ranging from minutes to hours reaching plasma concentrations up to  $1.7 \mu\text{M}$ . In particular, high frequency GnRH bursts should be considered when designing drugs competing with GnRH. In addition, the GnRHR internalizes slowly and is thus not a limiting factor for drugs to be effective. We hypothesize that (functional) antagonists targeting the GnRH receptor should have a long residence time to overcome the high frequency pulses and fast association kinetics of the endogenous ligand. This is particularly beneficial when chronic treatment is desired, e.g. for treatment of prostate cancer or endometriosis.

#### **Box 2: Alternative mechanisms involved in regulating ligand concentrations**

Neuropeptides are generally degraded by peptidases, and reuptake systems or binding proteins are often not involved in regulating free ligand concentrations. However, a few exceptions have been reported where alternative mechanisms are proposed to regulate high neuropeptide concentrations.

For instance, a binding protein has been discovered, called the CRF binding protein (CRF-BP), that binds CRF, Ucn1 and their associated peptides with high affinity [49]. This protein is broadly distributed throughout the brain and its predominant role is to bind and clear CRF from the blood. CRF-BP is also expressed in the liver and placenta where it is believed to modulate CRF levels and protect the body from increased plasma CRF levels, particularly during late stages of pregnancy [50].

Another exception is reported for cholecystokinin octapeptide (CCK8). The degradation of CCK8 by peptidases is much slower in comparison to other neuropeptides and therefore an alternative control mechanism was hypothesized. A highly selective uptake mechanism was reported that together with peptidases enables termination of CCK8 activity [51].

Taken together, ligand-binding proteins and reuptake systems, although rare, can play a role in regulating free neuropeptide concentrations and should therefore be considered regarding endogenous neuropeptide concentrations.

#### *Neuropeptide Y and Neuropeptide Y receptors*

Neuropeptide Y (NPY) is a 36 amino acid neuropeptide hormone that acts as a neurotransmitter in the central nervous system (CNS). NPY is the principal endogenous agonist at neuropeptide Y type 1 ( $Y_1$ ), type 2 ( $Y_2$ ) and type 5 ( $Y_5$ ) receptors. NPY is released in high frequency bursts upon stress stimuli (Box 1 and Figure 2) [39] and plasma concentrations are reported to be around 10  $\mu\text{M}$  [52]. NPY receptors belong to class A GPCRs and are coupled to  $G_i$  or  $G_o$  proteins [53]. NPY receptors and their endogenous ligands are involved in the control of appetite, inhibition of anxiety in the CNS, presynaptic inhibition of neurotransmitter release in the CNS and periphery, the modulation of circadian rhythm and pain transmission [54]. NPY receptors are mainly targeted to treat stress-related disorders but also in pain treatment, cancer and epilepsy [2].

The kinetic binding profile of endogenous neuropeptide Y ligands to human  $Y_1$ ,  $Y_2$ , and mouse  $Y_5$  receptors was extensively studied by Dautzenberg *et al.* At 22°C,  $^{125}\text{I}$ -NPY displays rapid association to  $hY_1$ ,  $hY_2$  and  $mY_5$  receptors (Table 1). Dissociation of  $^{125}\text{I}$ -NPY from the  $mY_5$  receptor and  $hY_1$  receptor provided residence times between 50 and 80 min. In contrast, minimal dissociation (approximately 20%) of  $^{125}\text{I}$ -NPY from both recombinant and endogenous  $Y_2$  receptor was observed after 24 h incubation. These findings indicate a pseudo-irreversible binding mode of NPY to the  $hY_2$  receptor [55], which adds complexity to drug development targeting the  $hY_2$  receptor.

Receptor internalization rates, as well as subsequent degradation or resensitization differ substantially between the different NPY receptor subtypes. Upon human NPY exposure, the  $Y_1$  receptor is rapidly internalized via clathrin-dependent endocytosis [56-58]. In addition, resensitization studies demonstrated that the  $Y_1$  receptor is rapidly recycled back to the cell membrane [56, 58, 59]. In contrast,  $Y_2$  receptors neither internalize nor desensitize [56], or only to a small extent with extremely slow internalization rates after prolonged agonist exposure [57, 60]. Internalization of  $Y_5$  receptors has not been extensively studied yet. However, it was reported that this receptor internalizes to a much slower extent than  $Y_1$  [60-62].

In conclusion, target kinetics for the NPY receptor subtypes vary greatly and NPY is released in high frequency bursts upon stress stimuli with plasma concentrations around 10  $\mu\text{M}$ . Rapid association and dissociation kinetics as well as internalization rates were observed for the NPY- $hY_1$  receptor complex. In contrast, while the binding kinetics of NPY to the  $hY_2$  receptor are similar to the  $hY_1$  receptor, internalization of  $hY_2$  is extremely slow.  $hY_5$  internalization has also been reported to be slow, with fast association and slow dissociation rates of NPY. Therefore, we postulate that drugs with fast binding kinetics are desirable when targeting the  $hY_1$  receptor, while fast association and slow dissociation kinetics are beneficial for  $hY_2$  and  $hY_5$  receptors. Slowly dissociating agonists are particularly interesting

for cancer treatment as they might accelerate receptor internalization [63] while a slowly dissociating antagonist could be beneficial for the treatment of obesity [64].

### CRF, Ucn1 and the CRF<sub>1</sub>R

Corticotropin releasing factor (CRF) and urocortin 1 (Ucn1) are hormones that are the primary CNS neuromodulators of the hypothalamic-pituitary-adrenal axis. CRF and Ucn1 regulate adrenocorticotrophic hormone (ACTH) secretion by the pituitary and are critical neurotransmitters in the neuroendocrine and behavioral response to stress [67]. CRF and Ucn1 are released in high frequency bursts in response to stress (Box 1 and Figure 2) [6]. Ucn1 is mainly expressed in cell bodies of the Edinger Westphal nucleus in the brain while CRF is more widely expressed in the CNS [68]. Plasma concentrations of Ucn1 are reported to reach up to 5  $\mu$ M, while maximal CRF concentrations are much lower (around 2 pM). During stress and/or pathological conditions levels of both endogenous ligands increase, which is most noticeable for CRF of which levels can go up to 0.5 mM [69-71]. CRF and Ucn1 exert their effect by activation of two CRF receptor subtypes, CRF<sub>1</sub> and CRF<sub>2</sub> receptors. These receptors both belong to the secretin-like class B subfamily of GPCRs and are primarily coupled to G<sub>s</sub> proteins. Several studies have indicated the involvement of the CRF system in human stress disorders, such as anxiety, depression and addiction [72].

Ligand binding kinetics studies of CRF and Ucn1 to the CRF<sub>1</sub> receptor are limited (Table 1). In an

early study De Souza *et al.*, studied the binding of <sup>125</sup>I-[Tyr<sup>0</sup>] CRF (<sup>125</sup>I-rCRF) to rat olfactory membranes at different temperatures [73]. This study demonstrated temperature-dependent <sup>125</sup>I-rCRF association to rat CRF<sub>1</sub> receptors with a  $k_{on}$  value of 0.52 nM<sup>-1</sup> min<sup>-1</sup> at 23°C. At this temperature, dissociation was reversible and monophasic, with a  $k_{off}$  value of 0.007 min<sup>-1</sup> and

#### Box 3: Examples of drugs targeting neuropeptide GPCRs with optimized binding kinetics.

As this was all studied in retrospect, these examples demonstrate the need for a better understanding of the kinetic profile of the target receptor and its endogenous ligands, in addition to the drug candidate.

**Candesartan** is a marketed angiotensin II subtype-1 (AT<sub>1</sub>) receptor antagonists for the treatment of hypertension. It has a residence time of 173 min (37°C) [11].

**Aprepitant** and **netupitant** are marketed NK<sub>1</sub>R antagonists to treat chemotherapy-induced emesis. Aprepitant has a residence time of 154 min (22°C) [11] and netupitant has been reported as an insurmountable antagonist with antagonistic effects lasting over 5 hours [65].

**Suvorexant** is a dual orexin receptor antagonist to treat insomnia. It has a residence time of 83 min for the orexin type 2 (OX<sub>2</sub>) receptor (room temperature) [66].

**Buserelin** is a GnRH peptide agonist used to treat hormone dependent diseases. It has a reported residence time of 111 min at 25 °C [29].

RT of 143 min while association and dissociation were more rapid at physiological temperature [73]. In contrast, [<sup>3</sup>H]-Ucnl association to the human CRF<sub>1</sub> receptor was slow and monophasic with a  $k_{on}$  value of  $0.06 \pm 0.024 \text{ nM}^{-1} \text{ min}^{-1}$  while dissociation was faster in comparison to CRF with a reported  $k_{off}$  value of  $0.017 \pm 0.007 \text{ min}^{-1}$  and RT of 58 min at room temperature [74].

CRF<sub>1</sub> receptors undergo rapid desensitization and internalization during continuous exposure to CRF or Ucnl [75]. Although Ucnl- and CRF-induced CRF<sub>1</sub> receptor internalization occurred to a similar degree, the receptor was shown to recycle and resensitize more efficiently after CRF stimulation [44]. Moreover, there is evidence of sex differences in CRF<sub>1</sub> receptor signaling and trafficking [76]. In male rats, a swim stress paradigm promoted CRF<sub>1</sub> receptor  $\beta$ -arrestin2 association, and internalization in LC neurons. However, in female rats stress-induced CRF<sub>1</sub> receptor- $\beta$ -arrestin 2 association remained low, and stress-induced CRF<sub>1</sub> receptor internalization was impaired [76]. Valentino *et al.* suggested that sex biases in both CRF<sub>1</sub> receptor coupling to G proteins, and CRF<sub>1</sub>- $\beta$ -arrestin 2 association makes females more sensitive to acute stress and less able to adapt to chronic stress [77].

To summarize, although ligand binding kinetics studies on the CRF<sub>1</sub> receptor are limited, it is likely that drugs targeting the CRF<sub>1</sub> receptor are competing with fast binding kinetics of CRF but slower binding kinetics of Ucnl. Additionally, CRF<sub>1</sub> receptor desensitization and internalization is fast in males but slow in females. These gender-specific internalization kinetics should be taken into account in the design of novel agonistic drugs targeting the CRF<sub>1</sub> receptor when treating e.g. depression. Antagonists targeting the CRF<sub>1</sub> receptor to treat e.g. addiction should have fast association and slow dissociation rates to overcome the slow dissociation kinetics of CRF and high plasma concentrations of both CRF and UCNI during stress. Considering that stress-related disorders often need chronic treatment, patients could benefit from slowly dissociating drugs.

## **Concluding remarks**

Drug-target association and dissociation rates play an important role in achieving safe and efficacious drug action *in vivo*. For example, numerous drugs have been proven in retrospect to be highly efficacious due to their slow dissociation rates (Box 3). Currently, drug discovery efforts are moving towards incorporating optimized binding kinetics prospectively. As many successful drugs on the market achieve their effects by competing with endogenous ligands, a better understanding of the pharmacological and physiological behavior of endogenous ligands and their receptors in the human body is crucial. This is particularly important for neuropeptides, since their release is generally pulsatile or in bursts consequent to stress stimuli, ultimately resulting in instant high local concentrations.

Moreover, to understand desired binding kinetics for the target of interest, insights into receptor internalization kinetics are beneficial, as this arguably terminates a drug's effect.

In this review we have presented evidence of varying ligand binding kinetics for the endogenous ligands of three exemplary neuropeptide receptors. In addition to the observed variability in ligand binding kinetics across these three exemplars, receptor internalization kinetics were also largely different for all three discussed neuropeptide receptors. Thus, collectively, this small case overview demonstrates a broad array of kinetic profiles for neuropeptide receptors, i.e. endogenous ligand release rates, binding kinetics and receptor internalization rates. Presently, drug discovery focusses mainly on characterizing drug candidates only, while the kinetic profile of the target receptor and its endogenous ligand(s) are most often neglected. Therefore, we believe it is a great opportunity for future drug research to include the kinetic profile of the target receptor and its endogenous ligand(s) to the drug discovery paradigm. Knowledge of these complete kinetic profiles could improve our understanding of desired binding kinetics and in turn lead to less attrition in (pre-) clinical phases of drug development and to more efficacious drugs.

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