Non-peptide ligands in the characterization of peptide receptors at the interface between neuroendocrine and mental diseases

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ABSTRACT

Hypothalamic receptors for neuropeptide Y, melanin-concentrating hormone, melanocortins and orexins/hypocretins as well as for the downstream signaling corticotrophic factor have been discussed broadly for their influence on food intake and reward but also on several psychiatric disorders. For the development of non-peptide ligands for the *in vivo* detection of alterations in density and affinity of such G-protein coupled (GPCRs) peptide receptors the requirements to affinity and pharmacokinetics have been shifted to thresholds markedly distict from classical GPCRs to dissociation constants < 0.5 nM, partition coefficients log P < 3.5 and transcellular transport ratios, e.g. for the permeability glycoprotein transporter, below 3. Nevertheless, a multitude of compounds has been reported originally as potential therapeutics in the treatment of obesity among which some are suitable candidates for labeling as PET or SPECT-tracers providing receptor affinities even below 0.1 nM. These could be unique tools not only for better understanding of the mechanism of obesity but also for investigations of extrahypothalamic role of "feeding receptors" at the interface between neuroendocrine and mental diseases.

1. INTRODUCTION

1.1. Hypothalamus as a Potential Target for Drug Development in the Treatment of Obesity and Related Mental Diseases
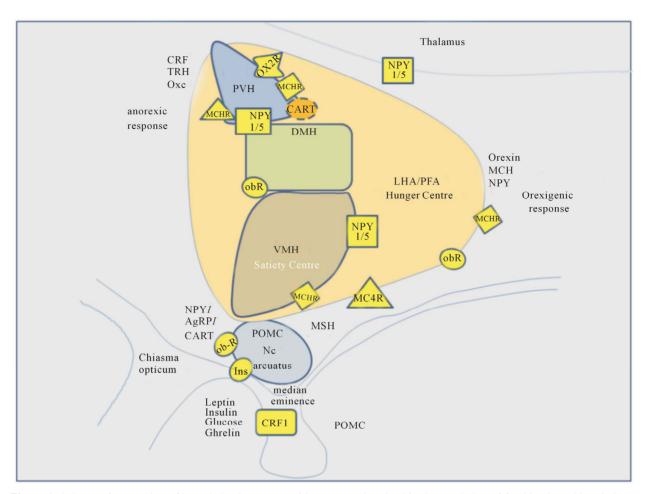
Among the huge number of central neuropeptide receptors the hypothalamic G-protein coupled receptors (GPCR) attracted early efforts of ligand development. Many regulatory peptides found in hypothalamic nuclei fulfill a dual function in the brain at the intercept between neuroendocrine metabolic and behavioral regulation [1-5]. One of the central motives for development of ligands of hypothalamic peptide receptors and hypothalamic-pituitary-axis (HPA) is the search for effective treatment strategies for endocrinological disorders and hormone-dependent tumor diseases [6]. Furthermore, effort is directed to potential therapeutics, prevention and diagnostics of obesity-related disorders. Many of feeding-related receptor agonists act not only in the hypothalamus but also in extrahypothalamic, telencephalic, mesencephalic and metencephalic areas thereby contributing to the regulation of behavior, as has been confirmed by numerous studies [5,7-17]. Consequently, rising attention is paid to ligands of feeding-related GPCRs as potential tools for the investigation of their role in behavioral disturbances.

The hypothalamic structural triad of the arcuate nucleus (Arc), paraventricular nucleus (PVH), and the ventromedial hypothalamus (VMH; referred to as "satiety" centre) as well as the neuronal relay formed by lateral hypothalamic area (LHA; in contrast referred to as "hunger" centre) all together with the perifornical area (PFA) represent a regulatory core unit of food intake [18] (see **Figure 1**).

The Arc, surrounding the third ventricle and adjacent to the eminentia mediana as the blood to brain gate to the hypothalamus for circulating hormones contains two discrete neuronal populations which act as first order neurons for insulin, leptin, ghrelin and other circulating metabolic signals. One population contains pro-opiomelanocortin (POMC) as precursor of melanocyte stimulating hormones (α-MSH, β-MSH, γ-MSH), β-endorphin as well as the cocaine-amphetamine-regulated transcript

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Figure 1. Scheme of expression of hypothalamic neuropeptide receptors involved in the regulation of food intake. Abbreviations: DMH dorsomedial hypothalamus. LHA lateral hypothalamic area, PFA perifornicular area, VMH ventromedial hypothalamus, PVH paraventricular nucleus, Ob-R leptin receptor, NPY1/5 neuropeptide Y1 and Y5 receptors, MCR melanocortin receptor, MCHR melanin-concentrating hormone receptor, CART cocaine- amphetamine regulated transcript receptor, AgRP Agouti regulated peptide, MSH melanocyte stimulating hormone, CRF1 corticotrophin releasing factor receptor 1. Scheme modified according to [18,101,251-254].

(CART) and is stimulated by leptin which inhibits in turn the other cell population containing neuropeptide Y (NPY) and agouti-related peptide (AgRP) [19]. MSH, β-endorphin and CART (endogenous ligands at melanocortin receptors, μ-opioid receptor and still non-identified CART receptors, respectively) [20] inhibit food intake per se similarly to other melanocortins e.g. via corticotrophin releasing factor (CRF1). NPY (via NPY1- and NPY5 receptors) and AgRP acting as endogenous melanocortin antagonist at MC4-R may enhance food intake [9,21]. The balance of neuronal activity in both cell populations is therefore a major regulating factor in feeding behavior.

Both neuronal populations project to the second order regions PVH (where oxytocin, CRF and, thyrotropin releasing hormone (TRH) are released) as well as to the LHA/PFA (releasing orexin and melanin concentrating hormone; (MCH) increasing food intake) [18]. This system serves as the long-term regulator of food intake. Activation of the neurons in PVH results in a catabolic state.

Short-term regulation of food intake is realized by secondary neurons in the nucleus tractus solitarii (NTS) and nucleus dorsalis nervi vagi. The secondary neurons of the NTS project via Nc. parabrachilis and the thalamic Nc. ventroposterior parvocellulares to the insular cortex.

For some of the GPCRs involved in this system receptor densities have been reported as shown in **Table 1**. The data suggest that especially in non-diencephalic regions of the brain the requirements to affinity, specificity and pharmacokinetics are high if *in vivo* molecular imaging of those receptors shall be made possible.

1.2. Peptidic Versus Non-Peptidic Receptor Ligand

For the characterization of neuropeptide receptors *in vivo* two profound observations have to consider. First, almost

all of these receptors exhibit relatively low receptor densities at their cerebral expression sites and second the uptake of peptidic tracers suitable for receptor labeling is possible per se but frequently limited due to inappropriate pharmacokinetic properties. Even small regulatory peptides with a molecular weight < 500 Da often show large differences in their potency to influence brain functions depending on the mode of application [22-27] and metabolic vulnerability [28,29].

Plasma half-life of peptide transmitters varies in a broad time range between several seconds and hours [23,24,30,31]. In general, the stability of endogenous peptides is difficult to predict and depends also from the presence and activity of cleavage enzymes in the blood or target regions.

Fifteen years ago it was postulated that neuropeptides released in the periphery either cannot cross the blood brain barrier (BBB) or can enter the brain only by very limited extent [32,33]. Although this hypothesis had been revised [27,34-36]), even today weak responses to exogenously applied peptidic receptor ligands are attributed to aggregation, poor enzymatic stability and various other limiting factors of BBB passage [23-25,27]. Further drawbacks to the suitability of peptidic compounds for therapeutic use are poor oral bioavailibility and high costs of production [37]. Alternative routes of drug delivery for instance transnasal administration can improve the accumulation of such drugs in the brain as has been shown for the somatostatin analogue octreotide [38]. However, this application route appears not suitable for nuclear medical purposes because of dosimetric limitations.

Although the affinity of peptidic tracers is frequently higher than that of non-peptidic ligands only few peptidic ligands developed to label brain receptors specifically could be introduced successfully into *in vivo* trials [15] due to limitations by the BBB. In consequence such tracers or drugs have been recommended and applied predominantly for therapy and diagnostics of peripheral tumors [6,15].

However, also a multitude of non-peptidic drugs and pharmacological probes [39] has been developed in parallel [39-41]. Some of those have been radio-labeled for *in vivo* imaging. Clinical trials have been performed for ligands at CRF1 receptors [39,42-47], at orexin receptors [48,49], at the NPY receptors [50,51], the opioid receptors [52], melanocortin -4-receptors (MC4-R) [53-55], melanin concentrating hormone receptors (MCH1-R) [56] and growth hormone secretagogue receptor 1 (ghrelin; GHS1-R) [57,58].

2. CRF-RECEPTORS

2.1. Endogenous Peptidic CRF Receptor Ligands

CRF (41 a.a.r.) is an early discovered prime coordinator of the synthesis of corticotrophin (ACTH; 39 a.a.r.) in the pituitary gland [59,60]. It is released from the neurons of the paraventricular region of the hypothalamus into the median eminence region which is one of the seven known circumventricular regions of the brain with interruption of the blood brain barrier [8,19,44,61,62]. Here, the paraventricular region is in close contact to the capillary plexus drained by long portal veins into the anterior pituitary a main target region of CRF [22].

Further endogenous peptides interacting with corticotrophin releasing factor receptors (CRF-R) are the urocortins 1 (Ucn1), (acting at CRF1-R and CRF2-R) Ucn2 and 3 (at CRF2-R) [63]. Two G-protein coupled receptor subtypes CRF1 and CRF2, which are members of the B-receptor family [64-69], as well as the CRF-binding protein [60] mediate the action of the transmitter peptides. CRF1-R as well as CRF2-R mainly couple to stimulatory G_s proteins [70,71] but trigger G_i and G_q dependent signal transduction mechanisms additionally [39,71].

CRFs-R are found wide spread throughout the brain but especially in stress responsive regions. They are expressed in relatively high densities in pituitary anterior (605 fmol/mg; human [72]) and intermediate lobe (200 fmol/mg; marmosets) [73]), but in rather moderate densities within prefrontal and cingulate cortex as well as in the subcortical part of the amygdala (centromedial nucleus) (60 - 150 fmol/mg; cynomolgus monkey) [62, 63,74] corresponding to the expression levels of the CRF1-R (see also **Table 1**). Hypersecretion of CRF has been postulated to be involved in the pathogenesis of several mood and anxiety disorders [5,15,75,76]. The CRF2-R has been found predominantly in the periphery [76] and is involved in the stress response, cardiovascular function and gastric motility [77]. In the rat the CRF2α variant of the CRF-R is expressed predominantly in the hypothalamus, while CRF2β-R has been observed in the heart and skeletal muscle [78,79].The splice variant in the human brain is the CRF2γ-R [79, 80].

An urotensin-like immunreactivity has been found in the region of the Edinger Westphal nucleus and colocalized with CRF2-R [81,82].

2.2. Non-Peptide CRF Receptor Ligands

A comprehensive overview on endogenous and synthetic peptides and on non-peptide ligands at CRF-Rs as well as pharmacophore development has been provided by Zorilla and Koob [15] together with a review of phase II/III clinical trials for CRF1-R antagonists in depression, anxiety and irritable bowel syndrome.CRF2-R antagonism has been described for some peptide analogues of antisauvagine-30 suitable for detection of gastrointestinal CRF2-R in mice but obviously not able to cross the BBB

Table 1. Densities of feeding receptors in brain tissues of different species obtained by autoradiographic and membrane binding studies (rodent, monkey, human) available in the literature (in fmol/mg protein and in one exception * in nM).

Receptor	Density (fmol/mg protein)	Region	Species	Method	References
CRF	605 200	Pituitary Pituitary	Human Marmoset	Autoradiography Autoradiography	[72] [72]
CRF	~150 ~140 ~60	Amygdala Cingulate cortex Hippocampus	Cynomolgus monkey	Membrane binding	[74]
CRF	490 ~200 ~300	Anterior lobe, pituitary Intermediate lobe Olfactory bulb	Rat	Membrane binding	[73]
CRF1	147-63 103-60	Occip. Cortex Cerebellar Cortex	Human	Membrane binding	[62]
NPY1	313 5*	Frontal Cortex Cortex	Human Rhesus Monkey	Membrane binding Membrane binding	[238] [124]
NPY2	458	Frontal cortex	Human	Membrane binding	[238]
NPY1/Y4	549	Forebrain	Rat	Membrane binding	[116]
MC4	1 - 4	Hypothalamus	Rat	Autoradiography	[239]
Orexin	40 - 135	Nucl. Accumbens, raphe nucl., CA3	Rat	Autoradiography	[187]
CART 61 - 102	49	Nc accumbens	Rat	Primary cell culture	[161]
MCH1	11	Brain	Rat	Membrane binding	[240]
MCH	7-fold increase	Brain	Mouse (fasted)	Brain slices, Antisense riboprobes, autoradiography	[199]

[83].

Whereas Zorilla and Koob [15] summarize CRF-R antagonists discovered since the late 1990s, Kehne & Cain [84] review experiences from animal models of mental disorders.

The first non-peptide CRF1-R antagonist was described in 1996 by Schulz *et al.* [85]. However, together with later reported ligands including antalarmin (1), NBI 27914, [86] DMP 904, CRA 1000 (2), CRA 1001 (3) SRA 12 5543A it shared the problem of high lipophilicity (**Table 2**) [87]. Chen *et al.* [88] reports for instance that CP 154, 526 (4) shows a volume distribution of 105 l/kg as well as a clog P of 8.43 and a half-life of 51 h [89] (**Figure 2(a)**). The lead compound NGD-98-2 presented in 2011 by Hodgetts *et al.* showed a log P of 6.3 [90].

Four non-peptide PET CRF1-ligands with affinities in the subnanomolar range have been described [47].

The radiosynthesis of a first CRF1-R PET ligand (5) was published for [^{18}F]FBPPA [91,92]. Together with FBPPA also the SPECT tracer [^{123}I]IBPPA (6) it was tested *in vivo* in Fischer rats (**Figure 2(a)**). Pharmacokinetic studies showed that the PET tracer was accumulated by 5.59% ID/g in the anterior pituitary. Fast wash out and poor specific binding in hypothalamus, amygdala and cerebellum were mainly accounted to its high log P > 6 [93]. The ratios pituitary/blood were for FBPPA 16.9:1 (5 min p.i.), 6.1:1 (60 min p.i.) and 5.6:1 (120 min p.i.).

Five minutes p.i. 4.28% ID/g of the SPECT tracer is presented in the pituitary declining to 1.98% ID/g until 120 min p.i. For [^{123}I]IBPPA the respective ratios pituitary/blood were 7.4:1 (5 min), 13:1(60 min) and 9.4:1 (120 min) [93] reflecting a much slower wash out than for FBPPA.

The group of Zuev *et al.* [93] reported that in analogues of FBPPA the introduction of polar groups into the 8-(6-methoxy-2-methylpyridin-3yl) ring resulted in a dramatic loss of potency. The observation that too strong hydrophilicity impaires ligand binding to CRF1-Rs brought up the N-fluoroalkyl-8-(6-methoxy-2-methyl-pyridin-3-yl) 2,7-dimethyl-N-alkylpyrazolo [1,5-α]1,3,5-triazin-4-amine analogue (7).The compound was found to combine useful binding characteristics with appropriate lipophilicity (IC$_{50}$ 6.5 nM, log D = 3.5 (clog P 2.3). and was announced as a candidate for testing as a potential PET tracer [93].

Further efforts to get compounds of lower lipophilicity resulted in 3-phenylpyrazolo [1,5-a]pyrimidines like NBI 30545, with a log P of 3.18 and an appropriate K$_i$ value,
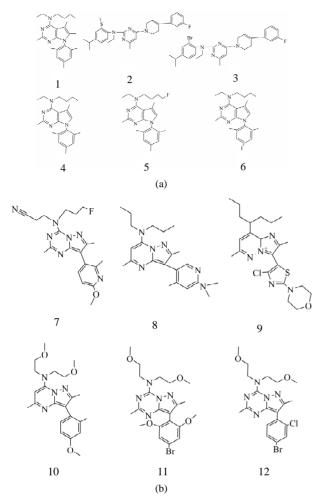
Figure 2. CRF1 receptor antagonists: (a) (1) Butyl-ethyl-[2,5,6-trimethyl-7-(2,4,6-trimethyl-phenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]-amine; (2) Ethyl-{4-[4-(3-fluoro-phenyl)-3,6-dihydro-2*H*-pyridin-1-yl]-6-methyl-pyrimidin-2-yl}-(4-isopropyl-2-methyl-sulfanyl-phenyl)amine; (3) (2-Bromo-4-isopropyl-phenyl)-ethyl-{4-[4-(3-fluoro-phenyl)-3,6-dihydro-2*H*-pyridin-1-yl]-6-methyl-pyrimidin 2-yl}amine; (4) Butyl-[2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]-ethyl-amine; (5) [2,5-Di-methyl-7-(2,4,6-trimethyl-phenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-yl]ethyl-(4-fluoro-butyl)-amine; (6) Butyl-ethyl-[7-(4-iodo2,6-di-methyl-phenyl)-2,5-dimethyl-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-yl]-amine; (b) CRF1 receptor antagonists: (7) 3-{(3-Fluoro-propyl)-[8-(6-methoxy-2-methylpyridin-3-yl)-2,7-di-methyl-pyrazolo[1,5-a][1,3,5] triazin-4-yl]-amino}-propionitrile. (8) [3-(6-Dimethylamino-4-methyl-pyridin-3-yl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-yl]-dipropyl-amine; (9) 3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-2,6-dimethyl-8-(1-propyl-butyl)-8aH-imidazo-[1,2-b]pyridazin-4-ylium; (10) Bis-(2-methoxy-ethyl)-[3-(4-methoxy-2-methyl-phenyl)-2,5-dimethyl-pyrazolo[1,5-a] pyrimidin-7-yl]-amine; (11) [8-(4-Bromo-2,6-dimethoxy-phenyl)-2,7-dimethyl-pyrazolo [1,5-a][1,3,5] triazin-4-yl]-bis-(2-methoxy-ethyl)-amine; (12) [8-(4-Bromo-2-chloro-phenyl)-2,7-dimethyl-pyrazolo [1,5-a][1,3,5] triazin-4-yl]-bis-2-methoxy ethyl)-amine. ChemDraw (Cambridgesoft.com) was used for verification of the IUPAC names.

as well similar compounds obtained by incorporation of

polar alkoxy groups [88]. Modifications of this lead structures allowed the identification of NBI 307775 (R121919) (8) (**Figure 2(b)**) and of a fluorinated analogue (3-(4 methyl-6-dimethylaminopyridin-3-yl)2,5-dimethyl-6-fluoro-7-dipropylaminopyrazolo [1,5-a] pyrimidine) as a potential PET tracer. However, first *in vivo* investigations in the baboon brain with the high affinity [^{11}C]R121919 were not successful, probably due to low receptor density of CRF1 subtype in these animals [62].

Promising drugs with appropriate physicochemical properties are also the imidazol pyridazines presented by Gehlert *et al.* [87] (MTIP (9) as well as by Richardson *et al.* (MPZP (10) (**Figure 2(b)**) [94]. MTIP has been described with a K_d of 0.22 nM, a log P of 3.55 as well as a bioavailability of 90% [15] (**Table 2**) and a distribution volume of 1.7 l/kg markedly improved in comparison to former candidates of *in vivo* imaging probes (e.g. the well known R121919 with 75.7 l/kg). Moreover, the intermediate plasma-half life of 3.3 h after p. o. administration is regarded as an advantage for potential therapeutic use.

MPZP showed a lower affinity (4.7 nM) but further improved lipophilic properties with log P of 2.95 [94].

More recently a Br-76 labelled non-peptidic CRF antagonist, (4-[^{76}Br]BMK-152) (11) (**Figure 2(b)**), has been described [95] which exhibited high affinity to rat and monkey frontal cortex (0.23 nM and 0.31 nM). Moreover, 4-[^{76}Br]BMK-152 showed an acceptable log P of 2.6 and is not a substrate of the P-glycoprotein (P-gp). During *in vivo* tests in monkey and rat it revealed binding of the tracer in brain regions consistent with the distribution of CRF -R.

3. NPY RECEPTORS

3.1. Endogenous Ligands of NPY Receptors

The 36 a.a.r. compound NPY is one of the most abundant neuropeptides in the brain. NPY-receptors (NPY1-R, NPY2-R, NPY4-R and NPY5-R) belong to the Class A (rhodopsin-like) GPCRs [96-100].

Insight into the network of NPY-Rs is given by many reviews e.g. by Kamiji & Inui in 2007 [101]. NPY has a dual effect on food intake. On one hand it is regarded as the most potent endogenous orexigenic agent [102] acting via NPY1-R and NPY5-R [103]. On the other hand it was found to mediate anorexia at the NPY2-R and NPY4-R [101]. In the Arc leptin depolarizes POMC neurons but hyperpolarizes NPY/GABA/AgRP neurons reducing the transmitter release from these terminals. The decrease of GABA is responsible additionally for the disinhibition of POMC neurons. Projections from NPY/AgRP and POMC/CART (rodents) neurons innervate neuron populations in the VMH, DMH, which also synthetize NPY, and to PVH which expresses NPY1- and NPY5-Rs [104]. The lateral hypothalamic area

Table 2. Partition coefficients log P and log D as well as molecular weights for selected neuropeptide receptor ligands. Bold and parenthesized numbers correspond to the number of the respective formula in the figures (ACD/ChemSketch and Scifinder software was the source of some of the data).

Compound	Peptide receptor	CAS registry	MW g/mol	Log P	Log D	References
CRA 1000 (**2**)	CRF1	226948-11-4	476.65	6.2	5.58	[241,242,250]
CRA 1001 (**3**)	CRF1	229346-94-5	509.45	7.2	6.6	[250]
MJL-1-109-2 (**12**)	CRF1		468.7	3.0		[243]
BMK-152 (**11**)	CRF1		494.38	2.6		[95]
SSR 125543A	CRF1	321839-75-2	483.04	7.10	7.11	[44,90]
NBI 27914	CRF1	184241-44-9	434.14	7.85	7.85	[244,245]
R 121919 (**8**)	CRF1	195055-03-9	380.53	4.79	4.75	[39,42,43]
CP 154526 (**4**)	CRF1	157286-86-7	364.53	6.63	6.15	[246]
MTIP (**9**)	CRF1	910551-43-8	420.0	3.55	3.55	[46,47,87]
MPZP (**10**)	CRF1	202579-76-8	398.5	2.95	2.93	[94]
[^{18}F]Y1-973 (**18**)	NPY1		472.63	3.2		[124]
UR MK 114 (**15**)	NPY1	1093677-54-3	529.6	3.619	2.45	[122]
JNJ 31020028 (**21**)	NPY2	1094873-14-9	565.68	2.98	2.83	[50,51]
JNJ 5207787 (**20**)	NPY2	683746-68-1	510.67	5.93	4.04	[108]
L 152,804 (**24**)	NPY5	6508-43-6	366.45	4.61	2.63	[129]
MK 0557 (**25**)	NPY5	328232-95-7	406.41	2.93	2.93	[103,131,247]
Almorexant (**31**)	Orexin A&B OX$_1$R, OX$_2$R	871224-64-5	512.56	5.89	5.89	[49]
SB649868 (**32**)	Orexin A&B OX$_1$R, OX$_2$R	380899-24-1	477.55	3.23	3.23	[48]
MCL 0129 (**30**)	MC4R	574001-66-4	546.76			[248]
T226296 (**46**)	MCHR1	331758-35-1	402.5	2.97	3.5	[56,249]

(LHA) releases melanin-concentrating hormone, orexin and NPY [105]. Its neurons communicate with the cerebral cortex where also afferent signals from the vagus nerve and the sympathetic nerve system arrive particularly via nucleus of tractus solitarii (expressing NPY2-R and NPY4-R) what allows the cortical coordination of feeding behavior [104].

Because of their potential role in obesity, NPY1-R and NPY5-R are the main targets for ligands in anti-obesity drug development. A multitude of antagonists for their identification has been reported [106]. With view to anxiety disturbances most attention has been paid to NPY1-R and NPY2-Rs [36]. mRNA of the four functional receptors has been found also in the amygdala supporting a role of NPY-Rs as prime candidates for the regulation of emotion, learning and fear memory [107]. This forces the interest in NPY-R ligands suitable for treatment of anxiety disorders. The NPY1-R receptor is also localized in cerebral cortex, caudate-putamen and thalamus [108], while NPY2-R is found primarily in hypothalamus, hippocampus, substantia nigra and cerebellum. Moreover, the NPY2-R has been found as pre- and postsynaptic receptor [109-111]. The distribution of NPY5-R has been verified by Weinberg *et al.* [112] by means of hybridization studies in mouse brain to be discretely limited to regions within the hypothalamus including nucleus suprachiasmaticus, anterior hypothalamus, bed nucleus striae terminalis and ventromedial nucleus. NPY-R signaling is mediated by G-proteins especially via pertussis toxin dependent $G_{i/o}$ proteins and decrease of cAMP formation. Hitherto among the six known NPY-Rs four (NPY1-R, NPY2-R, NPY4-R and NPY5-R) have been confirmed to couple to G_i-protein [113], whereas the existence of NPY3-R has been postulated but not demonstrated and the NPY6-R gene is not found functional in primates.

Moreover, several downstream signaling pathways mediate biological actions of NPY-Rs in the brain via Ca^{2+} channels or G-protein coupled inwardly rectifying

potassium channels (GIRK) [36,113,114] as well as mitogen activated protein kinase (MAPK). Different phospholipase C subtypes and IP_3 have been shown to be involved in signal transduction by NPY in different tissues and many questions on special regulatory chains also in brain cells remained unanswered even today.

3.2. Non-Peptide Ligands of NPY Receptors

The NPY1-R subtype was the major target of efforts in research groups of several companies which developed antagonists with relatively high affinity and selectivity as J-104870, J-115814, BMS-193885 (13), BMS205749 (14) (**Figure 3(a)**), SAR-135966 ,1229U91 (GW 1229 or GR 231118) [115-120].
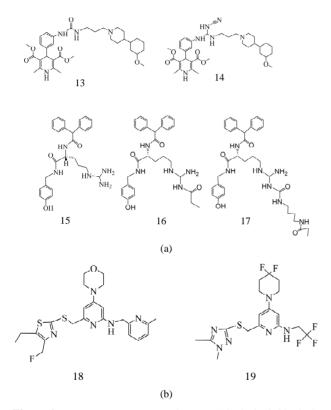

(a)

(b)

Figure 3. NPY1 receptor antagonists: (a) (13) 4-[3-(3-{3-[4-(3-Methoxy-cyclohexyl)-piperidin-1-yl]-propyl}-ureido)-phenyl]-2,6-dimethyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid dimethyl ester; (14) 4-{3-[(Cyanoamino-{3-[4-(3-methoxy-cyclohexyl)-piperidin-1-yl]-propyl-amino}-methyl)-amino]-phenyl}-2,6-di-methyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid dimethyl ester; (15) 5-(Diaminomethyl-amino)-2-diphenylacetylamino-pentanoic acid 4-hydroxy-benzylamide; (16) 5-[(Amino-propionylamino-methyl) amino]-2-diphenylacety-lamino pentanoic acid-4-hy-droxy-benzyl-amide; (17) 5-({Amino-[3-(4-propionylamino-butyl)-ureido]-methyl}-amino)-2-diphenylacetyl-amino-pentanoic acid 4-hydroxy-benzyl-amide. (b) NPY1 receptor antagonists: (18) [6-(5-Ethyl-4-fluoromethyl-thiazol-2-ylsulfanylmethyl)-4-mopholin-4-yl-pyridin-2-yl]-(6-methyl-pyridin-2-yl-methyl)-amine; (19) 6'-(81,5-Dimethyl-1H[1,2,4]triazol-3-ylsulfanyl-methyl)-4,4-difluoro-3,4,5,6-tetrahydro-2H-[1,4'] bipyridinyl-2'-yl]-(2,2,2-trifluoro-ethyl)-amine.

The first orally active Y1 antagonist was the pyrrolidine derivative SR 120819A [121]. Up to date, however, no ligands for the *in vivo* imaging of NPY1 receptors in clinical use for humans have been presented [51,103].

Keller *et al.* [122,123] and Weiss *et al.* [124] recommended the synthesis of ligands using substitution of argininamide type compounds with an acylguanidine entity at the guanidine as in BIBP 3226 (15) or the acylation of the guanidine group with propionic acid as in UR-MK114 (16) (**Figure 3(a)**) leading to high affinity (K_d 1.2 nM) and selectivity for NPY1-Rs. Additionally, these antagonists exhibit an excellent long-term stability and only slow radiolysis if storage is performed as TFA salt in ethanol. However, during longer periods of incubation at physiological pH the release of argininamide interferes with the efficiency of UR-MK114.

N^G-carbamoylation instead of alkanoylation can avoid this disadvantage [123]. Therefore, BIBP-3226 (15) was substituted with a N-propionylated aminobutylcarbamoyl moiety.The newly synthetized NPY1 ligand, UR-MK136 (17) (**Figure 3(a)**) showed a K_i value similar to that of UR-MK114 [123].

Recently, the presentation of the ^{18}F-labeled NPY1 antagonist Y1-973 (18) (**Figure 3(b)**) [124] was a breakthrough in NPY1 PET imaging. The tracer showed a IC_{50} of 0.13 nM for human Y1-R, a log P of 3.2 (**Table 2**) and a P-gp ratio of 1.3.These properties meet the requirements to peptide receptor ligands with $K_i < 0.5$ nM, log P < 3.5; and P-gp ratio < 3, which have been declared to be a generally necessary feature [120,124]. The authors emphasize the favourable kinetics in the brain, an accumulation in the striatum at 30 min with a binding potential of 1.7 and a high uptake also in cortical regions, whereas the uptake was moderate in thalamus and lowest in cerebellum as can be expected for a NPY1-R ligand Y1-718 (IC_{50} 17 nM) (19) was used for investigation of reversibility and specifity of Y1-973 binding [124].
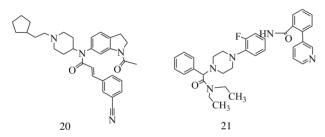
A first small-molecule Y2-R antagonist, JNJ-5207787 (20) (**Figure 4**), had been reported by Bonaventure *et al.* [108]. The compound showed a modest affinity and a receptor occupancy of 50%.

A further NPY2-R antagonist described by Seierstad *et al.* [125] and further characterized by Shoblock *et al.* [51], JNJ-31020028 (21) (**Figure 4**), possessed a high selectivity, an affinity close to that of BIIE-0246 [126] between 6 and 9 nM and a Hill coefficient of 1. Investigations with this ligand provided evidence that NPY2-Rs play rather a modulating role, but not a pivotal one in the occurrence of preponderance and obesity.
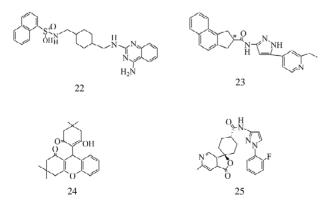
Also the role of NPY5-R has been discussed controversially. This was the case predominantly for its role in the occurrence of obesity in competition to NPY1.

CGP 71683 (22) (**Figure 5**) was the first NPY5-R antagonist reported in the literature [127,128] with a K_i of 1.4 and extremely high selectivity for NPY5-Rs.

Sato *et al.* [103] (23) (**Figure 5**) introduced the aryl pyrazole *N*-[5-(4-chlorophenyl)-1*H*pyrazol-3-yl]-2-indane carboxamide (K_i 59 nM) as a lead structure to inhibit the NPY5-R. The scaffold was substituted with a chiral 3,3-dihydropental[α]naphthalene moiety resulting in a novel high affinity compound (K_i 3.5 nM). However, originally developed as a therapeutic drug it was not included in clinical studies because it was not effective enough following oral application in rats.

Kanatani *et al.* [129] concluded from experiments with the orally active, selective NPY5 receptor antagonist (L-152,804) (24) (**Figure 5**) (K_i for displacement of PYY: 26 and 31 nM at human and rat NPY-Rs) that the NPY5-R contributes in part to the feeding response to NPY in their model. This contribution, however, was regarded as negligible. Recently, Nguyen *et al.* [130] using mice with individual NPY1- or NPY5-R knockout

or combined one demonstrated that food intake requires the concerted actions of both receptors, though compensatory mechanisms induced by a general knockout have to take into account.

A series of NPY5-R antagonists developed five years after the study by Kanatani *et al.* starting with the report on MK0557 (25) (**Figure 5**) [131] based on spiroindol scaffolds and achieved K_i values of 0.57 nM [132]. A combination of high receptor affinity and suitable brain/plasma ratio showed a spiroindol linked with a biphenyl. But with a log D > 4 and a poor bioavailability [132] it did not meet the criteria as a probe for *in vivo* receptor imaging. A further compound provided lower NPY5-R affinity (K_i 1.5 nM) but a log $D_{7.4}$ of 2.79 and was therefore used as ^{11}C-labeled radioligand. This tracer was not a substrate for the human P-gp transporter [132].

4. MELANOCORTIN RECEPTORS

4.1. Melanocortins, Agouti-Related Peptide and Their Receptors

Melanocortins, including ACTH and MSH, are characterized by the amino acid sequence His-Phe-Arg-Trp (HFRW) in their active core. The tetrapeptide motif has been recognized to be essential for their binding and agonistic activity at the melanocortin receptors (MCR) [9]. Such endogenous compounds are products of the cleavage of the 241 amino acid precursor pro-opiomelanocortin (POMC) by prohormone convertases (PC) acting in different parts of the pituitary gland as well as in the hypothalamus. Both subtypes of PC have been identified in anterior and intermediate pituitary. The expression of the distinct peptides has been suggested to be determined by the ratio between PC1 and PC2 (also referred to as PCSK1 or PC3 and PCSK2, respectively). PC1 in corticotroph cells supplies ACTH-related peptides (ACTH, ß-lipoprotein (LPH) and a K-peptide) in pars anterior distalis of the pituitary gland. In melanotrophs of the pars intermedia PC1 and PC2 reveal predominantly α-MSH-related peptides (α-MSH, corticotrophin like intermediate peptide; CLIP) as well as LPH-processed to ß-endorphin [55]. PC2 has been recognized to be responsible for the formation of the α-, ß- and γ MSH [133]. A former review [134] refers also to the less studied peptids γ$_3$-MSH and desacetyl- α-MSH.

Five receptors, MC1-R to MC5-R, are known as binding sites of the melanocortins. Two of them-the MC3-R and MC4-R -are localized also in the central nervous system. MC3-R is found mainly in the hypothalamus, cortex, thalamus and hippocampus [135,136] but also in kidney and gut. MC4-R has been observed in the hypothalamus, thalamus, hippocampus and other parts of the limbic system as well as in brainstem, with the highest

Figure 4. NPY2 receptor antagonists: (20) N-(1-Acetyl-2,3-dihydro-1H-indol-6-yl)-3-(3-cyano-phenyl)-N-[1-(2-cyclopentyl-ethyl)-piperidin-4-yl]-acrylamide; (21) N-{4-[4-(Diethyl-carbamoyl-phenyl-methyl)-piperazin-1-yl]-3-fluoro-phenyl}-2-pyridin-3-yl-benzamide.

Figure 5. NPY5 receptor antagonists: (22) N-[[4-[[(4-aminoquinazolin-2-yl)amino]methyl]cyclohexyl]methyl]naphthalene-1-sulfonamide; (23) 2,3 Dihydro-*1H*-cyclopental[a] naphthalene-2-carboxylic acid [5-(2-ethyl-pyridin-4-yl)-1*H*-pyrazol-3yl] amide; (24) 9-(2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-enyl)-3,3-dimethyl-2,3,4,9-tetrahydro-xanthen-1-one; (25) trans-N-(1-(2-fluorophenyl)-3-pyrazolyl)-3-oxospiro(6-azaisobenzofuran-(3H), 1'-cyclohexane)-4'-carboxamide.

affinity in the dorsal motor nucleus of the vagus nerve and spinal cord [137].

Important functions of the MC3-R are associated with the cardiovascular control as well as sodium and energy homeostasis. MC4-R is discussed predominantly for its direct role in the regulation of food intake and energy expenditure [138]. Mutations of the MC4-R have been shown to result in binge eating and obesity [11, 139-141]. MC3-R is presumed to be involved in regulation of food intake rather indirectly. Mutations induce a mild obesity related to an insulin-resistant phenotype [142]. ACTH and MSH act as agonists at the MC-Rs and mediate anorexic actions in normal individuals [20]. In obese individuals, however, the anti-obesity effect of agonists seems to be very limited, possibly because of downregulation of the melanocortin effects by the arrestin pathway [20].

MC-Rs have been found in contrast to many other seven-transmembrane-domain-receptor types to recognize not only endogenous agonists but also inverse agonists or endogenous antagonists [143,144].

One of these is agouti signaling protein (ASIP) which under normal physiological conditions antagonizes MC1-R effects [20,145] and controls skin pigmentation. It is a peptide triggering a switch in pigmentation from eumelanin to phaeomelanin [146]. The second antagonistic endogenous ligand, AgRP, acts at the hypothalamic MC4-Rs and MC3-Rs as has been shown in transgenic mice overexpressing AgRP. AgRP can be processed by PC1 resulting in a smaller peptide 6fold more potent at MC4-R than AgRP (131 a.a.r. (mice) and 132 a.a.r. (humans) [147]. ASIP (110 a.a.r.) and AgRP show similar CYS rich C-terminal domains which belong to the motif responsible for the interaction with MC-receptors. The cystine rich domains are presumed also to be responsible for a stereotypic tertiary structure defining a new structural class for vertebrate proteins [146] the cystine-knot peptides [148].

Only a small population of Arc neurons (3000 in mice) has been shown to be POMC positive but develops widespread extra-hypothalamic projections to brainstem, medulla and spinal cord [9,149] as second order neurons containing MC4-R and MC3-R.

In general the MC-Rs are regarded to be coupled to G_s protein signaling [20,21,150]. The MC4-R, however, is known also to couple to $G_{i/o}$ and G_q proteins influencing cAMP or Ca^{2+} release. Moreover, MC4-R activation has been shown to interact with insulin signaling via MAPK including the c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK1/2) [133,151, 152].

4.2. MC-R Antagonists

Most of the MCR ligands have been developed as agonists for the treatment of obesity and erectile dysfunction

[37,153] although the success in the diminution of overweight remained markedly behind the expectations. Peptide MC4-R antagonists as HS-014, HS-024, HS-131 and MBP-10 have been known for longer time as helpful research tools. Among these MBP-10 [153,154] has been described as the most interesting due to its high affinity (K_i 0.5 nM) and a selectivity to MC4-R 125 fold higher than to the MC3-R [37]. However, only few MC4-R antagonists are available as potential drugs or as probes for *in vivo* investigations. Whereas for peptide compounds vulnerability to peptidases, low brain permeability and high costs of synthesis are obstacles, in non-peptidic antagonists the affinity in relation to the density of the receptors is in general too low for the use in PET or SPECT imaging approaches. Bednarek and Fong provided 2004 [37] an overview on ligands available in the years 2002-2003 and Blankeney *et al.* [39] included in 2007 MC receptor antagonists in their reviews on neuroreceptor ligands. Potential candidates for *in vivo* imaging could be based on piperazine templates or on a 3-iodo-4-chloro-aminoguanidine wich achieved in binding studies K_i of 10 nM at MC4-R (compared to 0.5 µM at MC1-R, 5.8 µM at MC3-R, 4.9 µM MC5-R) [37]. Several of the tetrahydroisoquinoline (THIQ) derivatives with MC4-R antagonizing properties and relatively high affinity could also penetrate the blood brain barrier e.g. compound (26) with a log D of 1.1 and a K_i of 1.8 nM, or compounds (27) and (28) (**Figure 6**) with log D of 1.8
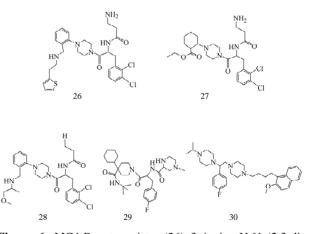

Figure 6. MC4-R antagonists: (26) 3-Amino-N-[1-(2,3-dichloro-benzyl)-2-oxo-2-(4-{2-[(2-thiophen2-yl-ethylamino)-methyl]-phenyl}-piperazin-1-yl-)-ethyl]-propionamide; (27) 2-{4-[2-(3-Amino-propionylamino)-3-(2,3-dichloro-phenyl)-propionyl]-piperazin-1-yl}-cyclohexanecarboxylic acid ethyl ester; (28) *N*-[1-(2,3-Dichloro-benzyl)-2-(4-{2-[(2-methoxy-1-methyl-ethylamino)-methyl]-phenyl}-piperazin-1-yl)-2-oxo ethyl] propionamide; (29) (2S)-N-[(1R)-2-[4-cyclohexyl-4-[[(1,1-dimethylethyl)amino]carbonyl]-1-piperidinyl]-1-[(4-fluorophenyl) methyl]-2-oxoethyl]-4-methyl-2-piperazine-carboxamide; (30) 1-[(1S)-1-(4-fluorophenyl)-2-[4-[4-(2-meth-oxynaphthalen-1-yl) butyl]piperazin-1-yl]ethyl]-4-propan-2- ylpiperazine.

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and K_i of 6.3 and 6.9 nM [155,156].

Wikberg & Mutulis [20] as well as Blankeney *et al.* [39] summarized derivatives (e.g. (29) (**Figure 6**) of the MC4-R agonist THIQ and the antagonist SHU9119 at the MC3-R and MC4R and emphasized further templates as potential scaffolds for MC4-R antagonists. Furthermore, a non-THIQ related low affinity piperazine had been reported in 2003 [157] as the first MC4-R antagonist. Modifications of this compound resulted in the synthesis of MCL-0129 (30) (**Figure 6**) which has been shown to improve stress induced depression and anxiety in rats [39, 54].

5. CART AND ITS POTENTIAL RECEPTOR

A receptor has been postulated also for CART peptides [158]. Rogge *et al*. proposed in 2008 [159] a signaling mechanism for such potential receptors. Due to the observation that signaling induced by CART can be inhibited by pertussis toxin a G_i/o protein coupled mechanism has been suggested but also activation of MEK and ERK ½ has been demonstrated in mouse pituitary cell lines [160]. CART itself is presumed to be involved in reward and reinforcement, feeding, stress and neuroendocrine control [161]. Active CART peptides occur by processing of a 89 and a 102 a.a.r. proCART peptide. The CART fragments 55 - 102, 62 - 102 and 42 - 89 [159] (with difference in the amino acid sequence of the active region in rats and humans) revealed biological activity also *in vivo* [162]. In humans has been observed only CART 42 - 89. The peptide can cross the BBB but is not subjected to mechanisms regulating saturation of its brain level [163]. The pro-peptides are predominantly expressed in hypothalamus, pituitary, adrenal gland and pancreas. A mutation has been found for the CART gene (Leu34Phe) in obese families [164,165] and the PACAP sequences 1 - 38 and 6 - 38 have been identified as low-affinity inhibitor of CART binding [166]. Moreover, the CART level has been observed to be markedly decreased in cerebrospinal fluid of patients suffering from dementia with Lewy bodies.

Rogge described for CART peptides a role in several parts of the stress axis including hypothalamus (retrochiasmatic nucleus, Arc, PVH), pituitary anterior and posterior, caudal raphe, rostral ventrolateral medulla, spinal cord as well as adrenal chromaffin cells [159].

6. OREXIN RECEPTORS

6.1. Endogenous Peptidic Ligands

Orexin A and B (33 and 28 a.a.r.) [167] also called hypocretin 1 and 2 according to the tissue where it has been identified [168] are both generated from pre-prohor-

mone-orexin (pp-orexin; 130 - 131 a.a.r.). This is expressed in the lateral hypothalamic area particularly close to the median eminence, DMH and PFA [169,170]. Stability and lipophilicity of orexin A exceeds that of orexin B. That is the reason for a higher plasma concentration of orexin A and its ability to cross the blood brain barrier in difference to orexin B [163].

Additionally to their intrahypothalamic actions on feeding behavior orexin releasing neurons project to extrahypothalamic structures like the histaminergic tuberomamillary nucleus and the basal forebrain, as well in the posterior and pontal locus coeruleus and the median raphe nucleus, substantia nigra and ventral tegmental area. Moreover, there are strong interactions with signaling by neurons of the ventrolateral preoptic nucleus (VLPO) involved in the regulation of the ascending arousal system [171]. VLPO has been characterized to be in close relation to nucleus suprachiasmaticus which is one of the most powerful pacemakers of the circadian sleep/wake cycle and known as the internal clock of the mammalian body. Orexin (hypocretin) has been recognized as a wake-promoting agent [172,173].

Enormous boost for the presumption of a role of orexin in the sleep/wake cycle came from the discovery of natural animal models of narcolepsy in dogs [174] starting with investigations in a dog model with excitation-stimulated loss of muscle tone caused by a deficiency of the OX2-R [175-177]. Humans suffering from severe disturbances of sleep/wake cycle as narcolepsy show low or absent orexin in the cerebrospinal fluid [178,179].

The number of orexin-containing neurons has been reported to achieve 3000 - 4000 in rats and 50,000 - 80,000 in humans [173,180]. Orexin receptors have been found in the highest density in locus coeruleus.

OX2-R shows a nearly equal affinity to orexin A and B (34 nM and 60 nM), whereas OX1-R has a higher affinity to orexin A (30 nM vs 2500 nM for Orexin B) [167,168]. For OX1-R signal transduction via $G_{q/11}$ proteins is suggested, whereas OX2-R can act via G_q as well as via G_i and G_s proteins [167, 181,182]. However, it is not finally clear if this description is true also in different kinds of target cells [180]. Nevertheless, OX-R actions are mediated also by Na/Ca exchanger and GIRK.
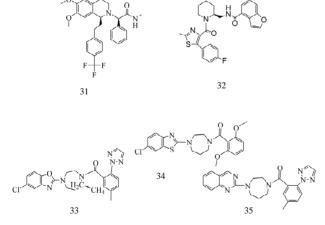
6.2. Non-Peptide OX-R Ligands

OX-R antagonists have been developed predominantly as therapeutics for insomnia and dual orexin antagonists (DORA) attracted the highest attention because they could effectively promote sleep. Meanwhile, however, DORAs as well as selective orexin receptor antagonists (SORA) have been investigated in clinical trials [183].

Almorexant (31) (ACT-078573), SB-649868 (32) and MK-4305 (33) (**Figure 7**) as well as Merck-DORA 1 and Merck-DORA-5 belong to the first group. Almorexant and SB-649868 improved the natural sleep architecture by increasing the time spent in REM and non-REM sleep phase in contrast to the GABA modulator zolpidem which reduces the time in these sleep stages [48,49]. Both were subjected to clinical trials (Almorexant to phase III and SB-649868 to phase I) but the latter was delayed in further testing by preclinical toxicological findings.

The diazepane compound MK 4305 (33) with K_i values of 0.55 and 0.35 nM at OX1-R and OX2-R [49,180, 184] as well as a molecular weight of 450.19 g/mol has a log P of 4.24 and is included in a clinical phase III study. A volume distribution of 2.6 (rat) and 0.8 l/kg b. wt (dog) lets expect rather modest lipophilic properties. MK 4305 has been developed using 7-methyldiazepane and triazolyl-benzamide as core unit and is only one in a series of diazepanes highly potent as DORAs (see (34), (35)) (**Figure 7**) [49,184].

The replacement of the benzoisoxazole moiety by benzopyrimidine and substitution in the 6-position by a fluorine atom resulted in a compound which was similarly to MK4305 not a substrate for the permeability glycoprotein (P-gp) and showed an excellent passive permeability. But the brain/plasma ratio between 0.4 and 0.6 excluded it from the list of candidates for *in vivo* imaging [49].

Although characterized by some favorable attributes the compound was shown to form electrophilic interme-

diates which can be trapped by GSH if incubated in human or rat liver microsomes. Further investigations suggest a role of the fluor atom in the bioactivation. There was no marked alteration of the reactivity to GSH in double fluorinated molecules [49].
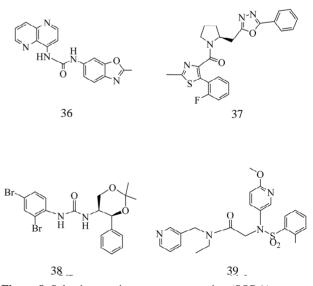
In general the DORAs available have higher affinities than the selective blockers. This is true also for the Merck-DORA-1 (0.2 nM and 3 nM for OX1-R and OX2-R, respectively) and Merck-DORA-5 (0.6 nM and 1.2 nM).

Nevertheless, selective OX1-R blockers have been found with SB 334867 (36) [185,186] and SB 674042 (37) and also OX2-R blockers have been characterized like JNJ-10394049 (38; 5 nM) and EMPA (39) (8 nM) [180,187] (**Figure 8**). Faedo *et al.* [182] subjected the newer orexin receptor antagonists not only to different *in vitro* activity tests but also to a sophisticated analysis of association and dissociation binding kinetics. The DORAs SB 649868 and MK6096 as well as the selective OX2-R antagonists ACT-078573, Roche-CP and JNJ10397049 were included. Short half-life of 0.19 min at OX1-R and 0.60 min binding to OX2-R where shown for JNJ10397049 associated with a surmountable antagonism with displacement of [^{3}H] ACT-078573.

7. MELANIN-CONCENRATING HORMONE RECEPTOR

7.1. Endogenous Peptidic Ligands

The mammalian melanin-concentrating hormone (MCH)

Figure 7. Dual orexin receptor antagonists: (31) 2-{6,7-Dimethoxy-1-[2-(4-trifluoromethyl-phenyl)-ethyl]-3,4-dihydro-1*H*-isoquinolin-2-yl}-*N*-methyl-2-phenylacetamide; (32) Benzofuran-4-carboxylic acid {1-[5-(4-fluoro-phenyl)-2-methyl-thiazole-4-carbonyl]-piperidin-2-ylmethyl}-amide; (33) (7R)-4-(5-chloro-1,3-benzoxazol-2-yl)-7-methyl-1,4-diazepan-1-yl]-[5-methyl-2-(2H-1,2,3-triazol-2-phenyl] methanone; (34) [4-(6-Chloro-benzothiazol-2-yl)-[1,4]diazepan-1-yl]-(2,6-dimethoxy-phenyl)- methanone; (35) (5-Methyl-2-[1,2,3]triazol-2-yl-phenyl)-(4-quinazolin-2-yl-[1,4]diazepan-1-yl)-methanone.

Figure 8. Selective orexin receptor antagonists (SORA) OX1-R: (36) 1-(2-Methyl-2,3-dihydro-benzooxazol-6-yl)-3-[1,5]naphthyridin-4-yl-urea; (37) [5-(2-Fluoro-phenyl)-2-methyl-thiazol-4-yl]-[2-(5-phenyl-[1,3,4]oxadiazol-2-ylmethyl)-pyrrolidin-1-yl]-methanone; OX2-R: (38) 1-(2,4-Dibromo-phenyl)-3-(2,2-dimethyl-4-phenyl-[1,3]dioxan-5-yl)-urea; (39) *N*-Ethyl-2-[(6-methoxy-pyridin-3-yl)-*o*-tolylsulfanyl-amino]-*N*-pyridin-3-yl-methyl-acetamide.

is a 19 a.a.r.cyclic peptide [188] which was discovered similar to the fish melanin-concentrating hormone (17 a.a.r.) earlier in extracts isolated from salmon pituitaries [189]. MCH supports the aggregation of melanin granules in melanophores resulting in a pale color of the skin. Its counterpart the melanocortin α-MSH disperses melanin pigments. MCH is formed by cleavage of a pre-prohormone which includes also amino-acid sequences of the neuropeptides E-I and G-E. Further splicing variants of the MCH gene can supply MCH gene overprinted polypeptide (MGOP)-14 and -17. MCH has been observed predominantly in lateral hypothalamus and zona incerta. Moreover, MCH has been detected in the periphery in testis and enteric neural system. In the peripheral tissue it is known to induce insulin hypersecretion and enhanced levels of the hepatocyte nuclear factor [190]. The absence of the peptide results in hypophagia and leaness [191]. MCH-Rs have been discovered in 1999 [192-197] and identified all over the brain but there are only few data available on the density of the receptors. These suggest a rather small cerebral receptor population compared with classical receptors well detectable by *in vivo* imaging methods. However, there are identified some regions with high expression of MCH-R mRNA [196-198] and it is known that fasting and obesity induce not only an upregulation of MCH but also of MCH1-R. The mechanism of this surprising response of the receptor is not completely understood [199,200]. The two receptor subtypes known in humans, MCH1-R and MCH2-R [201,202], are GPCRs. MCH1-R is acting via G_i/G_o and Gq proteins [197,203]. Actions of MCH2-R are mediated via G_q proteins [204]. MCH1-R mRNA levels have been described to be high in the olfactoric system, VMH, Arc and zona incerta [205,206]. but it has been found also in hippocampus, PVH, subiculum, basolateral amygdala and in the shell of the nucleus accumbens which are involved in learning, addiction, emotion and motivated behavior [196]. The presence of MCH2-R has been confirm in human hippocampus as well as in the amygdala and is discussed controversially for hypothalamic regions.[201,202,207]. Nevertheless, it has been not confirmed that MCH2-R is essentially involved in feeding behavior or neuroendocrine function [205].

7.2. Non-Peptide Ligands of MCH-Receptors

The observations that mice with overexpression of MCH1-R are obese, MCH1-R knockout animals become lean and can develop a hypophagia [208,209] and leaness may occur also in consequence to ablation of melanin-concentrating hormone neurons [210] accelerated the efforts in search for suitable antagonistic agents up to date. Among these GW 856464 [211], AMG076 [212]

and NGD-4715 [213] achieved phase 1 clinical investigations. Trials to optimize quinazolines [214-219], as (40) (**Figure 9(a)**), revealed GW 803430 (GW3430; [214]), ATC0175 and ATC0065 [215] which were shown efficacious in rodent depression models [197]. However, ATC0175 and ATC0065 are binding with high affinity not only to MCH1-R but also to subtypes of the 5 HT receptor [197] suggesting that the antidepressiv effect of the MCH1-R antagonist is not due to MCH1-R alone.
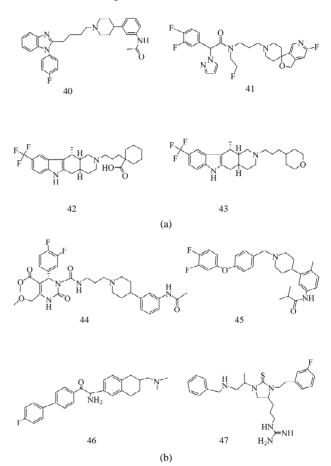

Figure 9. (a) MCH1 receptor antagonists: (40) *N*-[3-(1-{4-[1-(4-Fluoro-phenyl)-1*H*-benzoimidazol-2-yl]-butyl}-piperidin-4-yl)-phenyl]-acetamide; (41) 2-(3,4-difluorophenyl)-N-(3- (6-fluoro-1H-spiro[furo[3,4-c]pyridine-3,4'-piperidine]-1'-yl)propyl)-N-(2-fluoroethyl)-2-(1H-pyrazol-1-yl) acetamide; (42) 1-[3-(11-Methyl-9-trifluoromethyl-1,3,4,4a,5,6,11,11a-octahydro-pyrido [4,3-b] carbazol-2-yl)-propyl]-cyclohexane-carboxylic acid; (43) 11-Methyl-2-[3-(tetrahydro-pyran-4-yl)-propyl]-9-trifluoromethyl-2,3,4,4a,5,6,11,11a-octahydro-1*H*-pyrido [4,3-*b*]carbazole. (b) MCH1 receptor antagonists: (44) 3-{3-[4-(3-Acetylamino-phenyl)-piperidin-1-yl]-propylcarbamoyl}-4-(3,4-difluoro-phenyl)-6-methoxymethyl-2-oxo,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid methyl ester; (45) N-[3-[1-[[4-(3,4-difluorophenoxy) phenyl]methyl] piperidin-4-yl]-4-methylphenyl]-2- methylpropanamide; (46) 2-Amino-2-(6-dimethylaminomethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)-1-(4'-fluoro-biphenyl-4-yl)-ethanone; (47) {(4*S*)-1-[(1*S*)-2-(benzylamino)-methylethyl]-3-[2-(3-fluorophenyl) ethyl]-2-thioxoimidazolidin-4-yl} propyl) guanidine).

Furthermore, also aminopiperidine chromones [220], bicycle heptane derivatives [221], phenylpyridones [222] and benzazepine derivatives [223] recently have been reviewed by Johansson and Luthin [224,225]. However, no MCH1-R ligand is in use up to now for clinical imaging of cerebral receptors. The central reason for that should be the requirements arising to affinity and physicochemical properties by the low density of MCH-Rs in the brain. Moreover, the action of MCH1-R ligands as drugs for the treatment of obesity is frequently biased by interactions of the compounds with the hERG potassium channel (human ether-a-go-go-related gene channel) [226, 227], a channel associated with the long QT syndrome. Therefore, research for new therapeutically relevant selective MCH1-R ligands is steered in part by the necessity to find templates with warranty of low or no interaction with such channels.
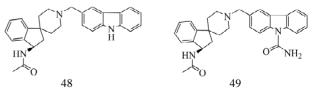
Several compounds notable as candidates for *in vivo* MCH1-R imaging have been reported by Suzuki *et al.* [228] including spiro-fluorofuropyridine derivatives with K_i values of 0.49, 0.15 nM and 0.09 nM. Beside the reduction of the unspecific actions on hERG recent reports provide extended pharmakokinetic data including not only lipophilicity, solubility and further distribution parameters but also data on microsomal stability to P-gp, as well as SAR influencing the potency of binding to the MCH1-R receptor. Suzuki *et al.* [229] tested the susceptibility of their compounds to P-gp in human MDR1- and mouse mdr1α transfected porcine renal epithelial cells. Agents revealing transcellular transport ratio above 3 in these experiments are regarded to be P-gp substrates. This is true for the most of the compounds investigated by Suzuki *et al.* [229]. That property provides an additional important feature of exclusion and allowed to decide for a spirofuoropyridine substituted with an ethyl group and a further fluorine (41) (**Figure 9(a)**). This antagonist showed a MCH1-R affinity of 0.09 nM and a log $D_{7.4}$ = 2.3. Two similar substances showed IC_{50} of 0.15 and 0.45 nM, respectively, but only one of it is not regarded as a P-gp substrate. Mihalcic *et al.* [205, 230] established the synthesis of isochonolin indols derived from alkaloid structures which were selected by means of an aequorin MCH1-R cellular assay using blockade of intracellular Ca^{2+} release by MCH. Derivatives with receptor affinities of 0.3 nM (42) and 0.6 nM (43) (**Figure 9(b)**) which can compete also with the affinity of the endogenous ligand of the MCH receptor (IC_{50} 1 nM and 5 nM, respectively) were reported. Souers *et al.* and Vasudevan *et al.* proposed [231-234] some high affinity compounds with quinazoline, piperidine scaffolds. These include SNAP-7941 (44) [235] with K_d of 0.18 nM, SNAP-94847(45) [236] and T226296 (46) (**Figure 9(b)**) [56,197] (**Table 2**). Especially SNAP-94847 has been described to show a more rapid onset of anti-de-

pressive action than a traditional antidepressant [236].

The thioxoimideazolimine TPI-1361-17 (47) (**Figure 9(b)**) has been selected among 800,000 compounds by data screening [188]. It could inhibit Ca^{2+} mobilization induced by 1 nM MCH with an IC_{50} of 6.1 nM and suppressed the food intake *in vivo* by 75% in rodents [188].

Particularly difficult is the identification of effective MCH2-R antagonists because the receptor had not been found in rodents. Nevertheless, Chen *et al.* [237] presented in 2012 a series of high affinity carbazol–derivatives with high selectivity for MCH2-R in comparison to MCH1-R.Two of the most effective compounds are (48) and (49) (**Figure 10**) with IC_{50} of 0.1 and 1 nM, respectively, in the CHO cells using Ca^{2+} FLIPR assay. (48) was obtained by replacement of hydroxy groups bound to the spiro- piperidine region by a N-acetyl group which can mimic the hydrogen donating character of the hydroxyl moiety [237]. Substitution of the carbazole with an urea group improved further the pharmacokinetic properties. (49) showed in MCH2-R binding assays a K_i rather moderate for a ligand which should be a suitable tool for neuroimaging. But the compound has a good selectivity versus MCH1-R and no interactions with cardiac potassium channels providing sufficient reasons, that the authors selected this substance for further pharmacological evaluation.

8. CONCLUSIONS

The general handicap for brain neuropeptide receptor *in vivo* imaging is primarily due to the relative low density of many subtypes of peptide receptors. Additionally, some of them, e.g. MCH2-R, are not inherently expressed in species typically used as animal models for the evaluation of receptor ligands, especially for development of radioligands for SPECT and PET of cerebral "feeding receptors". However, many reports on brain areas rich of neuropeptide receptor mRNA suggest the possibility to identify occasion-related changes of receptor expression if high affinity ligands are available. Furthermore, the pool of receptor ligands developed originally for therapeutic purposes and for labeling or blocking peripheral receptors revealed repeatedly compounds

Figure 10. MCH2 receptor antagonists: (48) (R)-N-(1'-((9H-carbazol-3-yl)methyl)-2,3-dihydrospiro[indene-1,4'-piperidine]-3-yl)acetamide; (49) (R)-3-((3-acetamido-2,3-dihy-drospiro[indene-1,4'-piperidine]-1'-yl)methyl)-9H-carbazole-9-carboxamide.

or templates appearing suitable for labeling with short-lived PET and SPECT isotopes and providing appropriate pharmacokinetic properties.

For the feeding receptors discussed here, at present, NPY-R ligands of the receptor subtypes Y1 and Y2 as well as CRF1-R ligands have the best perspective to become a diagnostic probe and to enter clinical applications due to relatively high receptor densities. MCH-R and MC-R as well as the hypothetic CART receptor should be the most challenging targets.

9. ACKNOWLEDGEMENTS

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