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Positron Emission Tomography (PET) Imaging of Opioid Receptors

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Positron Emission Tomography (PET) Imaging of Opioid Receptors

21

Aren van Waarde, Anthony R. Absalom, Anniek K. D. Visser,
and Rudi A. J. O. Dierckx

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Abstract

The opioid system consists of opioid receptors (which mediate the actions of opium), their endogenous ligands (the enkephalins, endorphins, endomorphins, dynorphin, and nociceptin), and the proteins involved in opioid production, transport, and degradation. PET tracers for the various opioid receptor subtypes are available, and changes in regional opioidergic activity have been assessed during both sensory and affective processing in healthy individuals and in various disease conditions such as chronic pain, neurodegeneration, epilepsy, eating disorders, behavioral addiction, and substance abuse. It is not always clear whether observed changes of tracer binding reflect altered release of endogenous opioids or altered opioid receptor expression. This issue may be resolved by studies in experimental animals that combine in vivo PET imaging with ex vivo immunohistochemistry. Some radioligands for opioid receptors have suboptimal kinetics (i.e., slow dissociation from their target protein) or can induce undesired side effects even at low administered doses (sedation, respiratory arrest). Yet, PET offers the unique opportunity of quantifying opioid receptor-mediated signaling in the living human brain. PET imaging has provided evidence for a link between opioid neurotransmission and peripheral immune activation.

21.1 Introduction

The term “opioid” is derived from opium: dried latex from the opium poppy (*Papaver somniferum*) which has been used since ancient times as an analgesic and recreational drug (Kalant 1997). An opioid is either a synthetic narcotic that has similar activities as opium or a naturally occurring peptide that has such activities. Several classes of endogenous opioids have been discovered, viz., the enkephalins

(Hughes et al. 1975), endorphins (Cox et al. 1976; Simantov and Snyder 1976), endomorphins (Zadina et al. 1997), dynorphins (Goldstein et al. 1979), and nociceptin (Meunier et al. 1995; Reinscheid et al. 1995). These messenger substances bind to G-protein-coupled opioid receptors in the brain, the spinal cord, and the digestive tract. Agonist binding to opioid receptors results in a decrease of the activity of adenylyl cyclase (Snyder and Pasternak 2003; Trescot et al. 2008; Waldhoer et al. 2004).

At least four broad subclasses of opioid receptors have been identified by the use of selective ligands (Dhawan et al. 1996). Assessment of antagonist potencies against the actions of various opioid agonists in different tissue preparations resulted in the identification of the mu, delta, and kappa subclasses (Lord et al. 1977; Martin et al. 1976). Later, mRNAs for these three subtypes were cloned and characterized (Chen et al. 1993; Evans et al. 1992; Kieffer et al. 1992; Yasuda et al. 1993). Mu opioid receptors (μ OR) are named after morphine, the alkaloid which is the active compound in opium (Martin et al. 1976). Kappa opioid receptors (κ OR) derive their name from the drug ketocyclazocine (Martin et al. 1976). Delta opioid receptors (δ OR) were initially characterized in the murine vas deferens (Lord et al. 1977). μ OR are β -endorphin preferring, κ OR dynorphin preferring, and δ OR enkephalin preferring (Minami and Satoh 1995). Nonclassical, nociceptin- or opiate-like receptors 1 (ORL1) bind nociceptin (Meunier et al. 1995; Reinscheid et al. 1995).

β -Endorphin binds with nM affinity to μ OR and δ OR (K_i about 1 nM) but with low affinity to κ OR ($K_i = 52$ nM) (Raynor et al. 1994). In contrast, dynorphin A has sub-nM affinity to κ OR ($K_i = 0.5$ nM), low affinity to μ OR ($K_i = 32$ nM), and negligible affinity to δ OR ($K_i > 1$ μ M) (Raynor et al. 1994). Enkephalins have sub-nM or nM affinity to δ OR and μ OR (K_i values 0.6–4 nM) but negligible affinity to κ OR ($K_i > 1$ μ M) (Raynor et al. 1994). Endomorphins are very potent and selective μ OR agonists ($K_i = 0.3$ – 0.7 nM) (Hackler et al. 1997). Stimulation of μ OR or δ OR generally results in analgesia and reward, whereas κ OR stimulation may have negative consequences, such as dysphoria and hallucinations.

Opioids and their receptors play important roles in a large spectrum of physiological processes, including learning and memory, reward, mood, appetite, circadian rhythms, sexual activity, pregnancy, locomotion, cardiovascular, gastrointestinal, renal and hepatic function, respiration, regulation of body temperature, and immunological response (Vaccarino and Kastin 2001). Well known is their involvement in regulating the sensation of pain; opiates are clinically employed to produce analgesia.

21.2 Opioid Receptor Ligands for PET: A Historical Overview

Several radioligands for PET imaging of opioid receptors have been prepared. These include subtype-selective and non-subtype-selective agonists and antagonists (see Table 21.1). Many comprehensive reviews on opioid receptor imaging were published between 2000 and 2008 (Frost 2001; Hammers and Lingford-Hughes

Table 21.1 PET probes for the opioid system

Name	Action	Abbreviation	Initial publication
[¹⁸ F]Cyclofoxy	Antagonist, μ OR, and κ OR	–	Pert et al. (1984)
[¹¹ C]Diprenorphine	Antagonist, non-subtype selective	DPN	Luthra et al. (1985)
[¹⁸ F]Diprenorphine	Antagonist, non-subtype selective	FDPN	Wester et al. (2000)
[¹¹ C]Buprenorphine	Mixed agonist/antagonist, non-subtype selective	BPN	Luthra et al. (1987)
[¹⁸ F]-FE-PEO	Full agonist, non-subtype selective	–	Riss et al. (2013)
[¹¹ C]Carfentanil	Agonist, μ OR	CFN	Dannals et al. (1985)
[¹¹ C]AH7921	Agonist, μ OR	–	Rafique et al. (2017)
[¹¹ C]Methylnaltrindole	Antagonist, δ OR	MeNTI	Lever et al. (1992)
[⁶⁴ Cu]TIPP conjugate	Antagonist, δ OR	–	Pirisedigh et al. (2017)
[¹¹ C]GR103545	Agonist, κ OR	–	Talbot et al. (2005)
[¹¹ C]EKAP	Agonist, κ OR	–	Li et al. (2019a)
[¹¹ C]FEKAP	Agonist, κ OR	–	Li et al. (2019b)
[¹¹ C]MeJDTic	Antagonist, κ OR	–	Poisnel et al. (2008)
[¹¹ C]JLY2795050	Antagonist, κ OR	–	Zheng et al. (2013)
[¹¹ C]JLY2459989	Antagonist, κ OR	–	Zheng et al. (2014)
[¹⁸ F]JLY2459989	Antagonist, κ OR	–	Cai et al. (2017), Li et al. (2018)
[¹¹ C]NOP-1A	Antagonist, ORL1	–	Pike et al. (2011)
[¹⁸ F]MK-0911	Antagonist, ORL1	–	Hostetler et al. (2013)

2006; Henriksen and Willoch 2008; Koeppe and Duncan 2000; Lever 2007; Ravert et al. 2004; Sprenger et al. 2005).

The first attempt at in vivo labeling of opioid receptors was made in 1975. Investigators at Johns Hopkins University administered a labeled opioid receptor antagonist, [³H]naloxone, to living rats. In the excised brain tissue of these animals, they observed a regional distribution of radioactivity which corresponded to the known distribution of opioid receptors in the rodent brain (striatum > hind-brain > cerebellum). Because of strong nonspecific binding of [³H]naloxone, acceptable target-to-nontarget ratios of radioactivity could only be reached after ex vivo washing of brain slices to remove nonspecifically bound tracer. Yet, this seminal study paved the way for all later imaging of neuroreceptors in the living brain (Pert and Snyder 1975).

The first successful PET probe for opioid receptors was prepared in 1984 (Pert et al. 1984; Channing et al. 1985). After intravenous administration of [¹⁸F]3-acetylcyclofoxy (3-acetyl-6-deoxy-6- β -[¹⁸F]-fluoronaltraxone), a regional distribution of radioactivity was observed which corresponded to the regional distribution of opioid receptors in the rat and baboon brain. Bound tracer in the baboon brain was completely displaced by the active (–)enantiomer of naloxone, whereas an

identical dose of the inactive (+)enantiomer (0.13 mg/kg) had no effect (Pert et al. 1984).

Since [^3H]naloxone showed a low ratio of specific-to-nonspecific binding, an antagonist with a higher affinity ([^3H]diprenorphine, [^3H]DPN) was prepared and successfully applied in autoradiography (review in (Frost 2001)). However, labeling DPN with ^{11}C proved difficult. Because of these difficulties, radiochemists focused their attention on other opioid ligands, and a method was developed to label carfentanil (CFN) with ^{11}C , using a carboxylic acid analog of carfentanil as a precursor (Dannals et al. 1985). The resulting tracer, [^{11}C]CFN, was used to acquire the very first images of opioid receptors in the human brain (Frost et al. 1985). At baseline, a regional distribution of radioactivity was observed which corresponded to the known regional densities of μOR . After treatment of the volunteer with naloxone (1 mg/kg), cerebral uptake of the tracer was strongly suppressed, indicating that [^{11}C]CFN binds to μOR in the living brain in vivo (Frost et al. 1985). A regional distribution of radioactivity consistent with μOR binding and blockade of tracer uptake by naloxone were also observed in the mouse brain. Thus, [^{11}C]CFN is suitable for studies of μOR in rodents (Saji et al. 1992).

In the same year that the first images of opioid receptors in the human brain were acquired, radiochemists developed a method for preparation of [^{11}C]DPN. The compound was labeled at the N-carbon atom of its cyclopropylmethyl group (Luthra et al. 1985). A study with [^{11}C]DPN in four healthy volunteers indicated that the tracer is rapidly washed out from brain regions with a low density of opioid receptors, whereas activity is retained in receptor-rich target areas. Eighty to ninety percent of tracer binding in target regions is naloxone reversible, suggesting that [^{11}C]DPN is suitable for studies of opioid receptors in man (Jones et al. 1988).

A different method to prepare [^{11}C]DPN was later published. That method involves [^{11}C]-O-methylation of 3-O-*t*-butyldimethylsilyl-(6-O-desmethyl) diprenorphine with [^{11}C]methyl iodide, followed by acidic deprotection of the reaction product (Lever et al. 1987). The advantage of this approach compared to the previously published method is its greater radiochemical yield.

The non-subtype-selective opioid ligand buprenorphine (BPN) has also been labeled with ^{11}C . The first published method involved the reaction of an *N*-(decyclopropylmethyl) precursor of buprenorphine with [^{11}C]cyclopropanecarbonyl chloride followed by reduction with lithium aluminum hydride (Luthra et al. 1987). Later, a more facile synthesis of [^{11}C]BPN was reported, in which the drug was labeled with ^{11}C at the 6-methoxy position, using a methylation reaction with [^{11}C]iodomethane (Lever et al. 1990). In a preclinical study in a baboon, the cerebral kinetics of [^{11}C]BPN and [^{11}C]DPN were directly compared. (–)Naloxone-sensitive binding of both ligands was observed in the striatum but not in the cerebellum. Uptake values and time courses in the target region were similar. The investigators concluded that [^{11}C]DPN shows better kinetics than [^{11}C]BPN since [^{11}C]DPN is cleared more rapidly from brain areas devoid of opioid receptors (Shiue et al. 1991). [^{11}C]BPN shows a regional distribution in the baboon brain consistent with regional opioid receptor density, whereas bound radioactivity in target areas can be displaced

by naloxone. Moreover, [^{11}C]BPN binding in the striatum shows a good ($\sim 10\%$) short-term test-retest variability (Galynker et al. 1996).

A non-subtype-selective agonist radioligand for opioid receptor imaging has become available. This compound, called [^{18}F]FE-PEO, binds with K_i values of 0.4–1.6 nM to all opioid receptor subtypes. The distribution of radioactivity in the rat brain corresponds to regional opioid receptor levels, but nonspecific binding is rather high ($\geq 33\%$ even in target areas). [^{18}F]FE-PEO is relatively slowly metabolized (60% parent in rat plasma after 3 h), and radioactive metabolites do not enter the brain (Riss et al. 2013).

A benzamide radioligand for μ -opioid receptors with agonist activity ([^{11}C]AH7921) was recently labeled with ^{11}C and tested in rats as a potential alternative to [^{11}C]CFN (Rafique et al. 2017). Although this agent showed several drawbacks, such as a low binding potential and entry of a radioactive metabolite in the brain, the authors claim that it may be used for quantitative imaging of μOR availability and competition with endogenous opioids.

The first successful radioligand for δOR was the antagonist $\text{N1}'$ -([^{11}C]methyl) naltrindole ([^{11}C]MeNTI). [^{11}C]MeNTI was synthesized by alkylation of 3-*O*-benzylnaltrindole with [^{11}C]methyl iodide followed by removal of the protecting benzyl moiety. [^{11}C]MeNTI binding in the brain of living mice was blocked (up to 75%) by naltrindole (δOR antagonist), but not by cyprodine (μOR antagonist) nor by U50,488 (κOR agonist). Regional tracer uptake showed a linear, close correlation to δOR densities known from autoradiography. These data indicated that [^{11}C]MeNTI binds selectively to δOR in the mouse brain in vivo (Lever et al. 1992).

An attempt to label the delta-1 subtype of δOR in the mouse brain with the selective ligand [^3H]benzylidenenaltrexone (BNTX) was only partially successful. Although some specific binding of the compound was observed in vivo and tracer uptake could be blocked with the appropriate compounds, ratios of specific-to-nonspecific binding were very poor (Lever et al. 1996). Fluorinated analogs of naltrindole ($\text{N1}'$ -fluoroethyl-naltrindole or BU97001 and $\text{N1}'$ -fluoroethyl-(14-formylamino)-naltrindole or BU97018) have been proposed for PET imaging. BU97001 has been tritiated and used successfully for autoradiography of the rat brain; it shows sub-nM affinity to δOR (K_d 0.42 nM) (Tyacke et al. 2002). However, PET data for [^{18}F]BU97001 have not yet been reported.

A ^{11}C -labeled *N*-substituted quinolinimide derivative with much higher subtype selectivity than [^{11}C]MeNTI has been tested as a potential ligand for in vivo imaging of δOR . The compound was rapidly degraded in both plasma and brain tissue after intravenous injection in rats, and specific binding could not be detected in vivo (Bourdier et al. 2007). A radiofluorinated δOR antagonist based on the Dmt-Tic pharmacophore has also been prepared. Although the compound showed significant binding to δOR in brain slices, it failed to cross the blood–brain barrier in living rats (Ryu et al. 2008). Three benzamide δOR agonists have been labeled with ^{11}C and subjected to preclinical testing. All compounds showed a very low brain uptake and no regional specific binding in mice. One of the compounds, called [^{11}C]SNC80, was also tested in a monkey. Results in this primate were similar to those seen

previously in mice, i.e., low blood–brain barrier permeability and a uniform distribution of radioactivity throughout the brain, indicating nonspecific binding (Pichika et al. 2010).

Recently, a conjugate of the δ OR antagonist TIPP was labeled with ^{64}Cu (Pirisedigh et al. 2017). This tracer was only tested by ex vivo autoradiography in rat brain sections. It showed a regional distribution corresponding to the known expression levels of the δ OR.

Initial attempts at developing subtype-selective radioligands for κ OR were not successful. ^{18}F -labeled analogs of U-50488 were prepared by fluoroalkyl substitution, but the labeling procedure resulted in a very strong (≥ 100 -fold) loss of affinity with reference to the parent compound (Chesis and Welch 1990). A successful agonist radioligand for κ OR, $[^{11}\text{C}]\text{GR89696}$, was finally prepared in 1999 (Ravert et al. 1999). The R enantiomer, later called $[^{11}\text{C}]\text{GR103545}$, shows an excellent hypothalamus/cerebellum ratio of 11.4 in the mouse brain at 90 min after injection, in contrast to the S enantiomer which is inactive (target/nontarget ratio = 1) (Ravert et al. 2002). Using subtype-selective opioid receptor ligands in blocking studies, the binding of $[^{11}\text{C}]\text{GR103545}$ was shown to be κ OR selective and saturable. Preclinical evaluation in baboons also indicated that $[^{11}\text{C}]\text{GR103545}$ is a promising tracer for imaging of κ OR. A regional distribution of radioactivity is observed that is consistent with the regional density of κ OR. Naloxone pretreatment reduces tracer uptake in all brain regions to the level observed in the cerebellum. In vivo binding of the tracer is stereoselective, and significant washout occurs within the time frame of a PET scan (Talbot et al. 2005). A one-pot radiosynthesis for preparation of $[^{11}\text{C}]\text{GR103545}$ with high specific radioactivity has recently been described (Nabulsi et al. 2011). Two other agonist radioligands for κ OR, FEKAP and EKAP, have recently been labeled with ^{11}C and evaluated in non-human primates (Li et al. 2019a; Li et al. 2019b). Both ligands show sub-nM affinity to κ OR, reasonable to excellent selectivity for κ OR over μ OR and δ OR, a fairly rapid metabolism in monkey plasma, fast and reversible kinetics in the brain and regional binding potential values consistent with binding to κ OR. Blocking and receptor occupancy studies were also performed, using the opioid drugs naloxone, LY2795050 and LY2456302. Thus, these ligands seem suitable for in vivo studies of cerebral κ OR.

Antagonist radioligands for κ OR have also been proposed. $[^{11}\text{C}]\text{MeJDTic}$ was prepared by methylation of the precursor JD Tic with $[^{11}\text{C}]\text{methyl triflate}$. In a pre-clinical study in mice, high uptake of the tracer was noted in target organs (lung, hypothalamus). Blocking and displacement studies with nonradioactive kappa, mu, and delta ligands suggested that $[^{11}\text{C}]\text{MeJDTic}$ binds specifically to κ OR in the brain of living mice in vivo (Poisnel et al. 2008). Yet, no follow-up studies with $[^{11}\text{C}]\text{MeJDTic}$ have been reported. A fluoropropyl derivative of JD Tic was later labeled with ^{18}F and evaluated in intact mice. This tracer failed because radioactive metabolites were formed that entered the brain and significant specific binding in target regions could not be detected (Schmitt et al. 2017). Another antagonist, $[^{11}\text{C}]\text{LY2795050}$, was evaluated in Sprague–Dawley rats, wild-type and κ OR knockout mice, and rhesus monkeys. Specific binding of the probe to cerebral κ OR

was observed; this binding was completely blocked by pretreatment of monkeys with naloxone (1 mg/kg) and dose-dependently inhibited by the selective κ OR antagonist LY2456302. Tracer metabolism in primates is moderate (40% parent remaining in monkey plasma at 30 min), and tracer pharmacokinetics appears favorable; thus, [^{11}C]LY2795050 is the first successful antagonist radioligand for κ OR (Zheng et al. 2013).

Still another κ OR antagonist, LY2459989, was labeled in recent years, both with ^{11}C (Zheng et al. 2014) and ^{18}F (Li et al. 2018). The two tracers show sub-nM affinity to κ OR and high selectivity for κ OR over μ OR and δ OR. They have been evaluated in rats and rhesus monkeys. A rapid uptake in the brain is observed and regional binding potential values are consistent with the known distribution of κ OR in primates. Pretreatment of animals with naloxone results in a homogeneous background radioactivity throughout the brain, and tracer uptake in target regions is dose-dependently reduced by the selective κ OR antagonists LY2456302 and unlabeled LY2459989. Thus, [^{11}C]- and [^{18}F]LY2459989 should also be considered as useful κ OR ligands.

The last subclass of the opioid system for which imaging probes have been developed is the nociceptin/orphanin or opiate-like receptor (ORL1). [^{11}C]Methyl-Ro64–6198 failed as a radioligand for this subtype since its *in vivo* binding in the mouse brain was largely nonspecific (Ogawa et al. 2001). A second probe, [^{11}C]CPEB, showed some saturable *in vivo* binding but lack of specificity to ORL1 (Ogawa et al. 2003).

The first successful ORL1 ligand was [^{11}C]NOP-1A, a specific antagonist with sub-nM affinity (0.15 nM). This compound was selected from a library of ORL1 antagonists developed by Eli Lilly & Co. (Pedregal et al. 2012). After intravenous administration of [^{11}C]NOP-1A to rhesus monkeys, a regional cerebral distribution of radioactivity is observed consistent with binding of the probe to its target. Brain uptake is reduced by 50–70% after pretreatment of animals with the ORL1 antagonist SB-612111 (Pike et al. 2011; Kimura et al. 2011). The regional distribution of [^{11}C]NOP-1A in the human brain resembles that in the monkey brain. Regional distribution volume of the tracer can be reliably estimated using a two-tissue compartment model with arterial sampling or Ichise's noncompartmental bilinear analysis (Lohith et al. 2012).

Another ORL1 antagonist for PET imaging has been developed by Merck. Their compound [^{18}F]MK-0911 shows a regional distribution in the monkey and human brain consistent with the regional expression of ORL1. Binding in the monkey brain is inhibited after pretreatment of animals with several structurally diverse ORL1 antagonists (MK-0584, MK-0337, MK-5757). Dose-dependent occupancy of ORL1 in the human brain by nonradioactive MK-5757 could be assessed with [^{18}F]MK-0911 and PET (Hostetler et al. 2013).

21.3 Characteristics of Widely Used Radioligands

21.3.1 Cyclofoxy

[^3H]Cyclofoxy (Fig. 21.1), the parent compound of [^{18}F]cyclofoxy, labels both μOR and κOR in autoradiography of the rat and guinea pig brain (McLean et al. 1987; Rothman and McLean 1988). The regional pattern of radioactivity in the rat brain after in vivo injection of [^3H]cyclofoxy corresponds to the regional distribution of these opioid receptor subtypes (Rothman and McLean 1988; Ostrowski et al. 1987), and brain radioactivity is reduced to background levels after pretreatment of animals with unlabeled naloxone (Ostrowski et al. 1987). K_i values of (–)cyclofoxy for μOR , κOR , and δOR in rat brain membranes are 2.6, 9.3, and 89 nM, respectively, whereas the (+)enantiomer is essentially inactive (K_i values $> 10\text{ }\mu\text{M}$) (Rothman et al. 1988). Consistent with these in vitro data, uptake of the radiofluorinated (–) enantiomer in the rat brain shows striking regional differences consistent with tracer

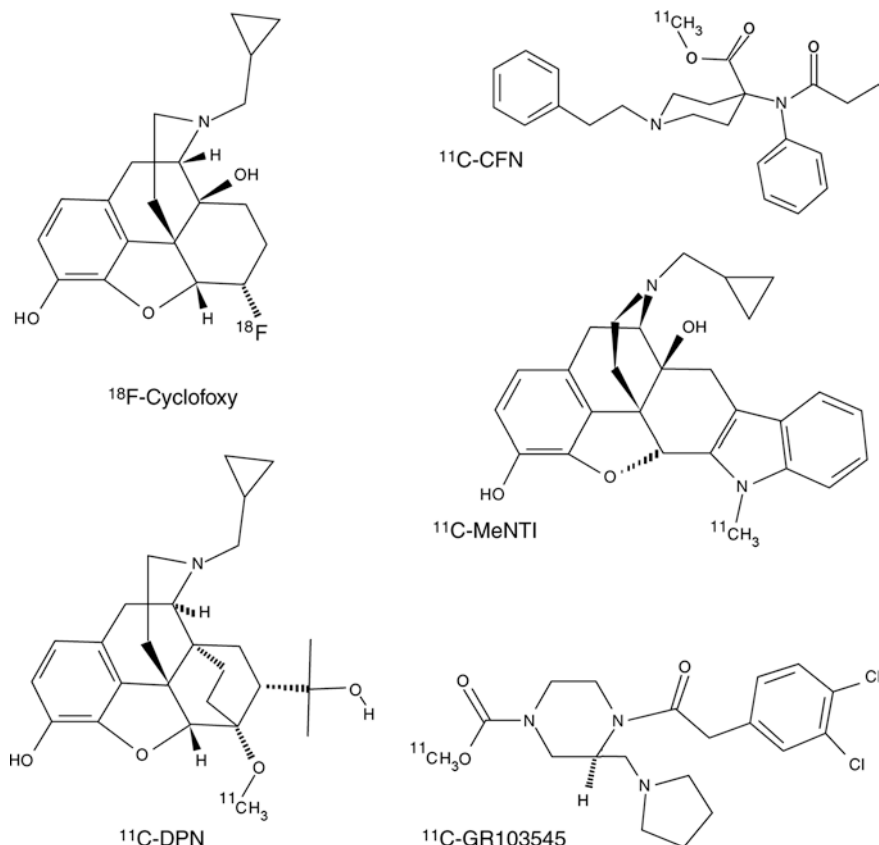


Fig. 21.1 Chemical structures of widely used opioid radioligands

binding to opioid receptors, whereas the tritiated (+)enantiomer is rather homogeneously distributed and shows low brain uptake. Pretreatment of animals with unlabeled (–)cyclofoxy reduces the uptake of [^{18}F](–)cyclofoxy in all regions to the level of the tritiated (+) enantiomer (Kawai et al. 1990). Thus, [^{18}F]cyclofoxy visualizes μOR and κOR in the living brain.

Kinetic analysis of [^3H]cyclofoxy uptake in the rat brain suggested an in vivo K_d of 2.1–5.2 nM in different brain areas and a B_{max} ranging from 1 pmol/g in the cerebellum to 78 pmol/g in the thalamus. Binding was reversible on a PET time scale (Sawada et al. 1991). In a follow-up study, a combination of bolus injection and constant infusion of [^{18}F]cyclofoxy was applied to estimate the receptor-ligand parameters in a condition of “true” tissue-blood equilibrium, which was reached after 60 min. K_d values of 1.4–2.9 nM were measured and B_{max} in target areas ranged from 15 to 74 pmol/g tissue. Rodent cerebellum could not be used as a reference region since the level of nonspecific binding in the cerebellum was different from that in other parts of the brain (Kawai et al. 1991). Bolus plus continuous infusion of [^{18}F]cyclofoxy was also advantageous in baboons, as it resulted in shorter scan times, a simplified protocol for blood sampling, and more accurate receptor measurements (Carson et al. 1993).

21.3.2 CFN (Fig. 21.1)

In vitro binding assays in human thalamic membranes indicated a K_d of 0.08 nM for [^3H]CFN (Titeler et al. 1989). In the rat brain, K_i values of 0.051, 4.7, and 13 nM were measured at μOR , δOR , and κOR , respectively (Frost et al. 1985). For the guinea pig brain, the corresponding values were 0.024, 3.28, and 43.1 nM (Cometta-Morini et al. 1992). In vitro competition studies using opioid ligands with a wide range of subtype selectivities confirmed that [^3H]CFN selectively labels μOR , in both the rat and human brain (Titeler et al. 1989). Thus, CFN is a potent and selective μOR agonist. Later in vitro binding studies on rat brain sections demonstrated that [^{11}C]CFN has higher affinity for the μ_1 subtype than for μ_2 . Small animal imaging studies in which the μOR ligand cyprodime and the μ_1 -subtype-selective ligand naloxonazine were used have indicated that the in vivo binding of [^{11}C]CFN involves only the μ_1 subtype, whereas binding to μ_2 receptors cannot be detected (Eriksson and Antoni 2015). Thus, [^{11}C]CFN images of the human brain may primarily show the distribution of the μ_1 subtype.

A study which applied compartment modeling for the analysis of [^{11}C]CFN binding in the human brain showed that the occipital cortex can be considered as a region with low or negligible receptor density. Target region-to-occipital cortex ratios of radioactivity at late time points can be used instead of ratios of k_3/k_4 when tracer kinetic modeling is not feasible, e.g., in clinical research protocols (Frost et al. 1989). However, Logan graphical analysis or a simplified reference tissue model approach can provide more accurate estimates of [^{11}C]CFN binding to μOR in the human brain than the ratio method (Endres et al. 2003). A test-retest study which assessed [^{11}C]CFN binding in the brain of healthy volunteers twice, on a

single day, indicated that both the regional two-tissue compartment model distribution volume (V_T) of the tracer and binding potential (BP_{ND}) are reproducible (variability <6 and $<10\%$, respectively) (Hirvonen et al. 2009).

Administration of [^{11}C]CFN can induce somnolence and sedation and, at higher dose, respiratory arrest. These pharmacological effects are related to the fact that CFN is an opioid agonist. The upper limit for the administered mass of CFN is about 0.1 $\mu\text{g/kg}$ (Frost 2001). [^{11}C]CFN binding in the human brain is affected by polymorphisms of the μOR gene. Carriers of the G allele (Asn40Asp variant, which may contribute to the development of alcohol dependence) have lower global binding potential than subjects homozygous for the A allele (Weerts et al. 2013).

Radiofluorinated analogs of carfentanil with subnanomolar affinities to μOR have been prepared (Henriksen et al. 2005a). *N*-[4-(methoxymethyl)-1-[2-(2-thienyl)ethyl]-4-piperidinyl]-*N*-phenyl-2-(\pm)-[^{18}F]fluoropropan-amide ([^{18}F]sufentanil) showed a high brain uptake in living mice and a distribution of radioactivity corresponding to regional μOR expression. The compound was rapidly metabolized in plasma (only 21% parent remaining at 40 min), but the metabolites appeared to not enter the brain in significant amounts (Henriksen et al. 2005b).

An updated (modernized) synthetic procedure for the preparation of [^{11}C]CFN (by reaction of a carboxylate precursor with [^{11}C]methyltriflate) has recently been described (Blecha et al. 2017). This procedure has the additional advantage that organic solvents could be replaced by ethanol.

21.3.3 DPN (Fig. 21.1)

Another widely applied radioligand for opioid receptor mapping is [^{11}C]DPN. [^3H]DPN binds with subnanomolar (K_d 0.22–0.23 nM) affinity to opioid receptors in the rat brain (Chang et al. 1981) and in the human frontal cortex (Pfeiffer et al. 1982). K_i values of DPN for μOR , δOR , and κOR in the rat brain are 0.20, 0.18, and 0.47 nM, respectively (Chang et al. 1981). Thus DPN is a non-subtype-selective opioid antagonist. Automated procedures for radiosynthesis of [^{18}F]FDPN and [^{11}C]DPN have been described (Schoultz et al. 2013; Fairclough et al. 2014).

Using RB101, an inhibitor of enkephalin-degrading enzymes, the in vivo binding of [^3H]DPN to opioid receptors in the mouse brain was shown to be sensitive to competition by endogenous enkephalins. Although RB101 is devoid of affinity to opioid receptors, the compound dose-dependently inhibits [^3H]DPN binding in the unstressed mouse brain up to a maximum of 30%. When mice were stressed by a warm-swim test, a greater inhibition of [^3H]DPN binding by RB101 was observed (maximally 45%). These effects of RB101 are probably related to increased levels of endogenous enkephalins (Ruiz-Gayo et al. 1992).

A validation study using [^3H]DPN in rats showed that compartment modeling of PET data with the cerebellum as reference region can provide accurate measurements of regional opioid receptor B_{max} and K_d , although K_d values estimated in vivo are an order of magnitude higher than those determined in vitro, probably due to diffusion gradients in tissue or protein binding of the radioligand (Cunningham

et al. 1991). However, the cerebellum cannot be considered as a region devoid of opioid receptors in human studies since μ OR and κ OR are expressed at low levels in this area of the human brain (Schadrack et al. 1999).

The kinetics of [^{11}C]DPN and [^{11}C]CFN in the brain of human volunteers have been directly compared (Frost et al. 1990; Villemagne et al. 1994). Binding of both ligands in the thalamus (an area containing mainly μ OR) was not significantly different, but [^{11}C]DPN showed significantly greater binding than [^{11}C]CFN in the striatum, cingulate, and frontal cortex. Dissociation of [^{11}C]CFN from its binding sites was observed, but dissociation of [^{11}C]DPN was hardly noticeable. [^{11}C]CFN binding reached a plateau, whereas [^{11}C]DPN uptake showed a steady increase (Frost et al. 1990). Total radioactivity in the human brain (SUV) and the ratio of total/nonspecific binding were twice as great after administration of [^{11}C]DPN than [^{11}C]CFN. The dose of naloxone required to fully block [^{11}C]DPN binding was ten times greater than that required for [^{11}C]CFN (Villemagne et al. 1994). Thus, [^{11}C]DPN appeared to label δ OR and KOR in addition to the μ OR which are selectively labeled by [^{11}C]CFN. In a later animal study, the sensitivity of [^{11}C]CFN and [^3H]DPN binding to endogenous opioid release was directly compared, opioid release being stimulated by administering amphetamine and methadone to rats. The binding of [^{11}C]CFN but not [^3H]DPN was significantly reduced after such challenges. In vitro binding and subcellular fractionation studies indicated that this may be due to the fact that [^{11}C]CFN binds preferentially to receptors on the cell surface, whereas [^3H]DPN binds to both surface and internalized receptors. Thus, [^{11}C]CFN may be a better ligand than [^{11}C]DPN for in vivo imaging of endogenous opioid peptide release (Quelch et al. 2014).

Both the regional density (B_{max}) and radioligand affinity (K_d) of opioid receptors can be assessed by applying a two-injection protocol, in which [^{11}C]DPN is administered at high (20–160 TBq/mmol) and low (1–3 TBq/mmol) specific radioactivities (Sadzot et al. 1991) or by a pulse-chase protocol (Jones et al. 1994a). Parametric images of [^{11}C]DPN binding can be prepared, using both conventional spectral analysis and rank shaping (Hammers et al. 2007a).

Radiofluorinated analogs of DPN have been prepared. *N*-(3-[^{18}F]Fluoropropyl)-*N*-nordiprenorphine (FPND) showed rather limited brain uptake (Bai et al. 1990) but a striatum/cerebellum ratio of 3.3 ± 0.7 at 30 min postinjection which was reduced to unity after pretreatment of animals with naloxone (1 mg/kg). The tracer was extensively metabolized (<25% parent remaining in blood at 30 min), but not significantly defluorinated (Chesis et al. 1990). 6-*O*-(3-[^{18}F]Fluoroethyl)-6-*O*-desmethyldiprenorphine ([^{18}F]DPN) showed a regional binding pattern in the human brain similar to that of [^{11}C]DPN (Wester et al. 2000). FDPN binds to μ OR, δ OR, and κ OR in rat brain slices like the parent tracer, DPN (Wester et al. 2000). A scan duration of at least 90 min is required for reliable estimates of tracer distribution volume (Boecker et al. 2005). If metabolite-corrected plasma data are used as input function, individualized metabolite correction should be performed since women metabolize FDPN significantly faster than men (Henriksen et al. 2006).

21.3.4 MeNTI

K_i values of MeNTI (Fig. 21.1) at δ OR, μ OR, and κ OR in guinea pig brain membranes are 0.02, 14, and 65 nM, respectively (Portoghese et al. 1990). Thus, MeNTI is a very potent and subtype-selective δ OR antagonist.

In the human brain, [^{11}C]MeNTI shows regional kinetics concordant with selective binding of the tracer to δ OR. Prolonged retention of radioactivity is observed in receptor-rich areas, whereas the ligand is rapidly washed out from nontarget areas of the brain. The regional distribution of radioactivity is closely correlated with local δ OR densities but not with regional densities of μ or κ binding sites. Tracer uptake is blocked after pretreatment of volunteers with naltrexone (up to 73%) (Madar et al. 1996).

[^{11}C]MeNTI shows irreversible binding characteristics in the human brain during a 90-min scanning period. A constrained three-compartment kinetic model is superior to other data-analytical techniques for quantification of the in vivo binding of the tracer to δ OR (Smith et al. 1999).

21.3.5 GR103545

The subtype selectivity of [^{11}C]GR103545 (Fig. 21.1) has been determined in HEK-239 cells expressing human κ OR, CHO-K1 cells expressing human μ OR, and CHO cells expressing human δ OR. K_i values at the three OR subtypes were 0.02, 16.2, and 536 nM, respectively (Schoultz et al. 2010). Thus, [^{11}C]GR103545 is an extremely potent and subtype-selective κ OR agonist.

In a preclinical study in awake rhesus macaques, [^{11}C]GR103545 showed a regional volume of distribution in the brain which corresponded to regional κ OR densities (Schoultz et al. 2010). In a more recent study, the use of a bolus-plus-continuous-infusion protocol was shown to permit estimation of both B_{\max} and K_d of κ OR in the monkey brain in vivo (Tomasi et al. 2013). The cerebellum was used as the reference region in kinetic analysis.

A low injected mass of [^{11}C]GR103545 is essential since this κ OR agonist can induce respiratory depression, reductions of heart rate, and sedation even at very low administered doses of 0.3 $\mu\text{g}/\text{kg}$. Based on the K_d value estimated for the radioligand in nonhuman primates, a dose of 1.4 μg (3.38 nmol) was estimated as an acceptable dose limit in human studies for subjects with a body mass of 70 kg (Tomasi et al. 2013). In a preclinical study in rats, [^{11}C]GR103545 was found to be suitable for estimation of the dose-dependent occupancy of the cerebral κ OR population by salvinorin A (Placzek et al. 2015).

A first-in-man study with [^{11}C]GR103545 has been performed. All subjects were scanned at baseline. Seven subjects were also scanned 75 min after oral administration of naltrexone and six subjects at two time points (1.5 h and 8 h) after intake of the selective κ OR antagonist PF-04455242. Drug treatment resulted in a decrease of tracer distribution volume (V_T) in all brain regions. A two-tissue compartment model with multilinear analysis method was identified as the method of choice for

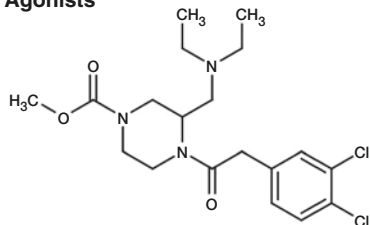
estimation of V_T . The in vivo affinity of the radiotracer for its target was estimated as 0.069 nM. [^{11}C]GR103545 seems suitable for quantification of regional κOR density and κOR occupancy in the human brain, although the radioligand has slow kinetics and variability in the estimated model parameters is rather high (Naganawa et al. 2014a).

21.3.6 LY2795050

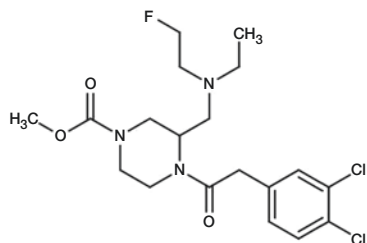
LY2795050 (Fig. 21.2) is an opioid antagonist with K_i values of 0.72, 25.8 and 153 nM at cloned human κ , μ and δ receptors (Zheng et al. 2013). In vivo competition studies in the monkey brain confirmed that [^{11}C]LY2795050 binds specifically and selectively to κOR . Although the in vivo selectivity of the ligand for κOR over μOR is smaller than that observed in vitro (7.6 rather than 36), the PET signal of a region expressing similar densities of κOR and μOR will predominantly (for 88%) reflect the presence of the κOR (Kim et al. 2013).

Blocking and kinetic modeling studies of [^{11}C]LY2795050 binding in the human brain indicated regional distribution volume (V_T) values corresponding to known regional densities of the κOR (Naganawa et al. 2014b). Since a reference region without specific binding is lacking in human brain, the authors proposed the use of a fixed value for V_T in cerebellum in model fits. This approach assumes that binding of the ligand in cerebellum is unaltered under the study conditions. A later

Agonists

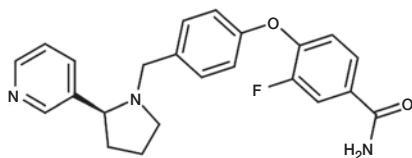


EKAP

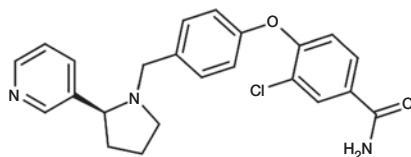


FEKAP

Antagonists



LY2459989



LY2795050

Fig. 21.2 Structures of some κOR agonists and antagonists that have been radiolabeled for PET imaging

publication examined the reproducibility of [^{11}C]LY2795050 scans in human brain, using a two-tissue compartment model fit with fixing of cerebellar V_T . The test-retest reproducibility of V_T in all brain regions was better than 10% with exception of the amygdala where it was 12%. In contrast to V_T , binding potential values showed a good reproducibility in regions with moderate or high receptor densities ($\text{BP}_{\text{ND}} > 0.4$) but a poor reproducibility in areas with small receptor populations (Naganawa et al. 2015).

An automated GMP procedure for preparation of [^{11}C]LY2795050 has been described (Yang et al. 2018).

21.3.7 LY2459989

LY2459989 (Fig. 21.2) is an opioid antagonist with K_i values of 0.18, 7.68, and 91.3 nM at cloned human κ , μ , and δ receptors (Zheng et al. 2014). Ex vivo biodistribution studies in rodents and imaging studies in monkeys have confirmed that [^{11}C]LY2459989 binds specifically to κOR . Pretreatment of monkeys with naloxone (1 mg/kg) results in a uniform distribution of radioactivity in the brain and tracer uptake is dose-dependently reduced after pretreatment of animals with the subtype-selective κOR antagonists LY2456302 and unlabeled LY2459989. The observed binding potential values of the tracer are high, and regional binding potential is an accurate reflection of known expression levels of the κOR (Zheng et al. 2014).

In a recent paper, sensitivities of the binding of [^{11}C]GR103545 and [^{11}C]LY2459989 in rat brain to competition by unlabeled κOR antagonists and agonists were directly compared. The binding potential values of both tracers were similarly affected when animals were pretreated with kappa antagonists (naloxone, naltrexone, LY2795050, JDTic, nor-BNI) and also after treatment of rats with the kappa agonists butorphan and GR89696. However, pretreatment of animals with the κOR agonists salvinorin A and U-50488 resulted in a strong decrease of [^{11}C]GR103545 binding, but did not affect the binding potential of [^{11}C]LY2459989 (Placzek et al. 2019). This surprising discrepancy suggests that agonists may interact in different ways with κOR and the target occupancy of some κOR agonists cannot be detected with [^{11}C]LY2459989.

21.4 PET Studies in Healthy Volunteers

21.4.1 Influence of Gender, Hormonal Status, and Age

[^{11}C]-CFN-PET has been applied for the study of changes of opioid receptor binding in the brain of women during the menstrual cycle. No significant differences in tracer binding were noted between the follicular and luteal phases of the cycle, but follicular levels of estradiol in the circulation were negatively correlated with [^{11}C]CFN binding potential in the amygdala and hypothalamus. These results, combined with measurements of luteinizing hormone pulse, suggested that μOR in the

amygdala modulate gonadal steroid hormone pulsatility, whereas circulating estradiol regulates μ OR function in the brain (Smith et al. 1998). Neuroimaging has shown that black cohosh, a herbal extract used to treat menopausal symptoms, affects the endogenous opioid receptor system. Significant increases in [^{11}C]CFN binding potential (10–61%) were noted in the cortical areas, thalamus, and nucleus accumbens of the brain of postmenopausal women (Reame et al. 2008). In the thalamus and nucleus accumbens, similar effects were observed after estrogen replacement therapy (Smith et al. 2006).

A PET study with [^{11}C]CFN, involving 50 subjects, indicated that the cerebral binding of [^{11}C]CFN is affected both by gender and by age. Binding potential of the radioligand increased with age in the neocortex and putamen. Women showed higher [^{11}C]CFN binding than men of the same age in several cortical and subcortical areas; however, in postmenopausal women, μ OR binding in the thalamus and amygdala declined to levels lower than in age-matched men (Zubieta et al. 1999). A PET study with [^{18}F]cyclofexy confirmed that opioid receptor binding in the thalamus of females is lower than in males, both in healthy aged subjects and in patients with Alzheimer disease (Cohen et al. 2000). MRI-based partial volume correction is required for the accurate assessment of receptor densities in small structures of the human brain. Applying such correction to [^{11}C]CFN data for the left temporal cortex, binding of the radioligand appeared to increase with age (0.9% per year), consistent with B_{max} values from postmortem autoradiography. Without partial volume correction, the effect of aging could not be observed (Bencherif et al. 2004a).

Correlations between regional serotonin transporter and μ OR binding were examined in a PET study involving 21 healthy volunteers. The subjects were first injected with the 5-HT transporter tracer [^{11}C]MADAM. When radioactivity from the first injection had decayed, the μ OR tracer [^{11}C]carfentanil was injected and a second PET scan was made, with the subject's brain in exactly the same position in the camera. Voxel-based correlation analysis was used to identify brain regions with positive correlations between serotonin transporter and μ OR binding. Significant correlations were observed in anteromedial thalamus, dorsal anterior cingulate cortex, dorsolateral prefrontal cortex, amygdala, left parietal cortex, and medial temporal cortex. This finding may indicate that in these regions, endogenous opioids are modulating serotonin release from nerve endings (Tuominen et al. 2014).

A PET study with the κ OR ligand [^{11}C]LY2795050 in 27 healthy volunteers reported opposite sex differences in κ OR availability as were reported for total opioid receptor binding with [^{11}C]CFN. Men had significantly higher V_T values than women in several brain regions (Vijay et al. 2016).

21.4.2 Feeding

An interesting PET study in ten healthy male volunteers examined whether feeding releases endogenous opioids in the brain and if the magnitude of this response is related to the pleasure of eating. Each volunteer was scanned three times with [^{11}C]CFN: (i) after an overnight fast, (ii) after an overnight fast followed by a

pleasant meal (preferred type of pizza), and (iii) after an overnight fast plus the consumption of an unpleasant meal (nutrient drink). Feeding was associated with a significant release of endogenous opioids (a decrease of radioligand binding potential throughout the brain), but this release was more pronounced after the unpleasant than after the pleasant meal. Thus, opioid release appears to be not directly related to the subjective pleasure but rather to metabolic and homeostatic responses of the body to food consumption (Tuulari et al. 2017).

21.4.3 Personality Traits

Several PET studies have explored relationships between opioid receptor availability in the human brain and personality traits. Using the tracer [^{18}F]DPN, German investigators scanned 23 healthy male volunteers under resting conditions. Subsequently, the subjects were psychologically tested for novelty seeking, harm avoidance, reward dependence, and persistence. A significant correlation was detected between the score for reward dependence (a personality trait predisposing for addictive behavior) and tracer binding potential in the bilateral ventral striatum with nucleus accumbens (Schreckenberger et al. 2008).

A later study from the USA involved scanning of 19 young healthy male volunteers with the μOR ligand [^{11}C]CFN and PET. Subjects were scanned both at baseline and during receipt of a painful stress challenge (infusion of hypertonic saline solution in the left masseter muscle). High scores for impulsiveness and low deliberation scores were associated with significantly higher baseline μOR availability and greater stress-induced endogenous opioid system activation, measured as a reduction of [^{11}C]CFN binding potential. These differences were noted in several brain areas (prefrontal and orbitofrontal cortices, anterior cingulate, thalamus, nucleus accumbens, and basolateral amygdala). Thus, μOR binding potential appears to predict an individual's vulnerability to risky behaviors, such as the development of substance abuse (Love et al. 2009).

A study from Finland measured brain μOR availability with [^{11}C]CFN under resting conditions in 22 healthy volunteers: 10 individuals scoring in the upper and 12 individuals scoring in the lowest quartile for harm avoidance. A high score for harm avoidance (particularly in the subscales shyness with strangers, fatigability, and asthenia) was associated with high μOR availability (or low endogenous μ -opioid drive) in the anterior cingulate cortex, ventromedial and dorsolateral prefrontal cortices, and anterior insular cortex. Thus, high μOR availability may be related to vulnerability for affective disorders (Tuominen et al. 2012). In a later study from the same group, 49 healthy volunteers were scanned with [^{11}C]CFN, and the avoidance and anxiety dimensions of their attachment were measured with the Experiences in Close Relationships-Revised scale. In this study, the avoidance dimension was found to be negatively (not positively) correlated with μOR availability (i.e., [^{11}C]CFN binding potential) in the thalamus, anterior cingulate cortex, frontal cortex, amygdala, and insula, whereas the anxiety dimension was not correlated with μOR availability in any brain region. The authors suggested that

inter-individual differences in μ OR availability may be related to differences in avoidant attachment style (Nummenmaa et al. 2015). In a study from the following year, measured [^{11}C]CFN binding potentials were compared to individual differences in behavioral dispositions when subjects encountered signals of reward and harm. Sensitivity of the behavioral activation system but not the behavioral inhibition system was found to be positively associated with [^{11}C]CFN binding potential in frontal cortex, amygdala, ventral striatum, brainstem, cingulate cortex, and insula. Particularly the score for “fun seeking” was strongly associated with μ OR availability (Karjalainen et al. 2016).

In an American PET study with [^{11}C]DPN in 12 healthy subjects, a high score of neuroticism was found to be associated with high opioid receptor binding in the right anterior insula. On the other hand, a high score of extraversion was associated with low tracer binding in the left posterior insula (Rodman et al. 2017).

21.4.4 Affective Responses

The opioid system appears to play an important role in the regulation of affective responses. An initial PET study examined [^{11}C]CFN binding and blood flow (uptake of ^{15}O -water) in the brain of 12 healthy male volunteers, which viewed either aversive images (facial mutilation, wounds, dead bodies) or neutral images (common objects, benign scenes, normal faces). Higher baseline μ OR binding potential in the left temporal pole of the subjects was associated with lower blood flow in this region during presentation of the aversive emotional stimuli. This finding was interpreted as evidence for an inhibitory or anxiolytic role of the opioid system in limbic regions of the brain (Liberzon et al. 2002).

A second study examined healthy female volunteers, using [^{11}C]CFN and PET. Sustained neutral and sadness states were elicited by the recall of an autobiographical event associated with these emotions. Sustained sadness caused an increase of μ OR availability in the rostral anterior cingulate, ventral pallidum, amygdala, and inferior temporal cortex which was interpreted as reflecting a reduction of the release of endogenous opioids due to deactivation of opioid neurotransmission (Zubieta et al. 2003).

Whereas sustained sadness in healthy volunteers is associated with a reduction in the release of endogenous opioids, positive emotion appears to be associated with an increase of that release. An interesting study from London scanned 25 healthy male volunteers with [^{11}C]DPN and PET, both during positive mood induction (sexually oriented video clip, listening to favorite music, reading positive statements, unexpected gift of 30 GBP) and during neutral mood (nature movie, listening to unknown classical music, reading neutral statements, no gift). Significant reductions of [^{11}C]DPN binding were noted in the hippocampus during positive mood induction, and the magnitude of positive mood change was negatively correlated with DPN binding in the amygdala (Koepp et al. 2009). An interesting PET study with [^{11}C]CFN evaluated the response of the μ -opioid system to social feedback. Eighteen subjects selected at least 40 profiles of preferred sex-individuals from a

database of 500. During a subsequent scanning session with [^{11}C]CFN, images of the faces of these individuals and their responses to the question: Would you like me? (Definitely no, or very likely yes) were presented to them on a computer screen (35 min of acceptance followed by 35 min of rejection, or vice versa, in counterbalanced order). During a separate scanning session with [^{11}C]CFN, the same subjects were scanned at baseline. The human faces on the screen were then replaced by gray blocks and in stead of feedback, the screen said: “non applicable.” Social rejection was found to be associated with significant opioid release in the ventral striatum, amygdala, midline thalamus, and periaqueductal gray, a response that may be aimed at reducing the experience of social pain. This interpretation was supported by the observation that a higher score for the character trait resiliency was associated with greater opioid release in the amygdala, periaqueductal gray and subgenual anterior cingulate cortex (ACC), whereas reduced negative feelings during rejection were associated with greater opioid release in the pregenual ACC. In contrast to social rejection, acceptance resulted in activation of opioid release in the amygdala and anterior insula but deactivation in the midline thalamus and subgenual ACC. Opioid release in the left ventral striatum during acceptance was associated with the desire for social interaction. Thus, neurotransmission via μOR may be involved in the preservation and promotion of emotional well-being of human subjects in their social environment (Hsu et al. 2013).

A PET study with [^{11}C]CFN examined the effect of social touch on the activity of the endogenous opioid system. Eighteen male volunteers were scanned twice with this tracer. During the baseline scan, the subjects were left alone, whereas during the social touch scan, their partners were present and were caressing them in a non-sexual way. The scans were separated by an interval of 2 h, and the order of the scans was counterbalanced, i.e., in half of the subjects, the baseline scan was made first, whereas in the other half, it was made last. The subjects reported pleasant sensations during the social touch scan, and the binding potential of the tracer was increased in various regions of the cortex (frontal, cingulate, insular), the thalamus, and the striatum, indicating deactivation of the endogenous opioid system (Nummenmaa et al. 2016).

Another study from the same group focused on the impact of social laughter on the opioid system. Twelve male volunteers were scanned twice with [^{11}C]CFN. Before the test scan, their closest friends were present in the room and they were watching laughter-inducing comedy clips together, but before the baseline scan the subjects spent 30 min alone in the testing room. After the laughing session, the binding potential of the tracer was decreased in the thalamus, caudate nucleus, and anterior insula. In cingulate and orbitofrontal cortex, the magnitude of this decrease was correlated to the rate of social laughter. These data suggest that endogenous opioids are released after social laughter (Manninen et al. 2017).

In a study with the PET tracers [^{11}C]CFN and [^{11}C]raclopride, regional μOR and D_2R availabilities were quantified in the brains of 35 healthy adult females. During subsequent fMRI sessions, the subjects watched movie scenes with varying emotional content, ranging from very pleasant to vary scary, and regional increases of cerebral blood flow were quantified. The availability of μOR in the brain was found

to be negatively correlated to fMRI-quantified blood flow responses to arousing scenes in amygdala, hippocampus, thalamus, and hypothalamus. Measured binding potentials of D₂R were not correlated with arousal or pleasantness in any brain region. Thus, emotional arousal appears to be regulated by the μ OR system (Karjalainen et al. 2019).

A PET study with the κ OR ligand [¹¹C]EKAP in a group of 18 healthy middle-aged volunteers examined the relationship between social status (measured with the Barratt Simplified Measure of Social Status test) and tracer volume of distribution in various areas of the brain. Social status was found to be inversely correlated to regional κ OR levels in amygdala, anterior cingulate cortex, caudate, frontal cortex, hippocampus, pallidum, putamen, and ventral striatum, i.e., in regions related to reward and aversion, whereas in other brain regions, no correlation was observed (Matuskey et al. 2019). This finding was interpreted as evidence for the hypothesis that κ OR may mediate the negative effects of social behaviors.

21.4.5 Physical Exercise

Long-distance runners have often reported to enter a state of euphoria while running, commonly referred to as “runner’s high.” In order to examine possible mechanisms underlying this phenomenon, ten athletes were scanned with [¹⁸F]DPN and PET, both at rest and after 2 h of endurance running (in random order). By SPM2 analysis, reductions of tracer binding were noted in several brain areas after exercise. Reported levels of euphoria were inversely correlated with [¹⁸F]DPN binding in the prefrontal/orbitofrontal cortices, anterior cingulate cortex, bilateral insula, parainsular cortex, and temporoparietal regions. These findings support the idea that running is associated with increased release of endorphins, resulting in positive mood changes (Boecker et al. 2008).

A more recent PET study with [¹¹C]CFN in seven healthy male subjects performing cycling exercise has indicated that the pattern and emotional impact of μ OR activation in the human brain is dependent on the intensity of the workload. Subjects performed either heavy or severe exercise. After both forms of exercise, reduced tracer binding was noted in several brain regions (particularly insular cortex and cerebellum), indicating activation of the opioid system. In heavy exercise, such decreases were correlated with increased positive sensations. However, in severe exercise, they were correlated with negative sensations. In severe but not in heavy exercise, an increase of tracer binding was noted in the pituitary gland, indicating deactivation of the μ OR system in that organ. This last finding may be related to the development of fatigue and exhaustion in exercising subjects (Hiura et al. 2017). Similar findings were reported in a later PET study with [¹¹C]CFN. In that study, 22 healthy subjects were scanned on 3 different occasions: after rest, a 60 min session of moderate exercise and a 60 min session of high-intensity exercise, using a bicycle ergometer. Moderate exercise did not result in significant changes of μ OR binding, although increased euphoria was associated with decreased μ OR availability. High-intensity exercise resulted in a significant decrease of μ OR binding in thalamus,

insula, orbitofrontal cortex, hippocampus, and anterior cingulate, i.e., in brain regions involved in pain, reward, and emotional processing. These regional decreases were associated with increased negative emotions, such as irritation, exhaustion, and dissatisfaction (Saaniyoki et al. 2018a).

Since the endogenous opioid system is involved in the rewarding aspects of both food and physical exercise, a combined PET-fMRI study in 24 healthy lean male volunteers examined the interaction between endogenous opioid release following exercise and anticipatory food reward. Two [^{11}C]CFN-PET scans were made of each subject on separate days: after 1 h of rest and after 1 h of moderate-intensity aerobic cycling exercise. Immediately after each PET scan, a fMRI scan was made during which images of palatable food and of nonpalatable items (cars) were presented to the subjects. The order of the rest and exercise scans was counterbalanced among the participants, and all participants fasted 3 h before each PET scan. Changes of μOR availability after exercise showed a large variability among subjects. A larger exercise-induced decrease of μOR binding was associated with larger increases in anticipatory food reward responses (food-induced increases of cerebral blood flow) following exercise. Thus, differences in μOR activation following exercise may contribute to inter-individual variation in food craving and food consumption (Saaniyoki et al. 2018b).

21.4.6 Pain

Opioid receptor expression ([^{11}C]DPN or [^{18}F]DPN binding) is high in projections of the medial and part of the lateral pain system of the human brain (Baumgartner et al. 2006; Jones et al. 1991). The lateral network is believed to be associated with the sensory aspects and the medial network with the affective aspects of pain perception. Reductions in receptor availability have been observed in healthy volunteers suffering pain. These data can be interpreted as decreases of regional receptor availability because of release of endogenous opioids, although internalization or downregulation of opioid receptors can also occur. Released opioids may mediate antinociception.

Sustained masseter muscle pain in 20 healthy volunteers was associated with significant declines of the binding of [^{11}C]CFN in several cortical and subcortical brain regions (Zubieta et al. 2001). When capsaicin was applied to the dorsal side of the left hand of eight healthy volunteers, a pain-related decrease in [^{11}C]CFN binding was measured in the contralateral thalamus (Bencherif et al. 2002). In eight healthy volunteers who received painful heat stimulation through the right forearm, a significant reduction of [^{18}F]DPN binding was noted in the limbic and paralimbic areas of the brain, including the rostral anterior cingulate cortex and the insula (Sprengr et al. 2006a).

The duration of pain-induced changes in μOR binding potential has been examined in a PET study with [^{11}C]CFN. Three subsequent 90-min scans were made in 14 healthy male volunteers; the first scan consisted of two 45-min baseline intervals, the second scan involved a 45-min period of sustained muscle pain followed by a

45-min baseline interval, and the third scan consisted again of two 45-min baseline intervals. Robust decreases of [^{11}C]CFN binding were noted in several cortical and subcortical regions during the pain challenge. However, these changes did not persist in a subsequent scan. Pain-induced alterations of opioid release and/or μOR internalization are therefore short lasting (Scott et al. 2007a).

In a study involving 14 male and 14 female healthy individuals subjected to sustained masseter muscle pain (infusion of hypertonic saline), men demonstrated larger declines of [^{11}C]CFN binding in the anterior thalamus, ventral basal ganglia, and amygdala. Conversely, women showed greater declines of the PET signal in the nucleus accumbens. Thus, opioid receptor-mediated antinociceptive responses show gender differences (Zubieta et al. 2002).

Opioid release is also involved in placebo analgesia. In a PET study with [^{11}C]CFN, healthy subjects were scanned under four different conditions: painful skin heat with placebo cream, painful skin heat under control conditions, nonpainful warmth with placebo cream, and nonpainful warmth under control conditions. In all cases, the volunteers were told that the placebo cream was a powerful analgesic. Placebo treatment resulted in significant decreases of [^{11}C]CFN binding in several brain regions known to be involved in pain and affect; thus, endogenous opioid release appears to be part of the mechanism by which expectancies regulate the perception of pain (Wager et al. 2007). A later study from the same group examined placebo and nocebo effects, i.e., the subjects expected the placebo cream to either ameliorate or exacerbate their pain. Placebo and nocebo effects were found to be associated with opposite opioid and dopaminergic responses. A placebo activated opioid neurotransmission in the anterior cingulate, orbitofrontal and insular cortices, nucleus accumbens, amygdala, and periaqueductal gray, whereas dopaminergic neurotransmission was activated in the ventral basal ganglia including the nucleus accumbens. Nocebo responses consisted of a deactivation of opioid and dopamine release. The magnitude of opioid and dopamine activation was correlated with the anticipated and subjectively perceived placebo effect (Scott et al. 2008). However, a subsequent [^{11}C]CFN-PET study in a large number of healthy volunteers ($n = 48$) indicated that a priori expectations (anticipations) are not correlated with placebo-induced decreases in pain ratings during placebo administration. Subjects with higher expectations showed greater μOR activation in the dorsolateral prefrontal cortex after placebo administration, but this did not correspond to subjective analgesic effects. The largest placebo responses were noted in individuals with low expectations and high subjective effectiveness. Activation of μOR in anterior cingulate cortex was correlated with the effectiveness of the placebo. In subjects with high expectations but low reported effectiveness of the placebo, placebo administration resulted in a nocebo, hyperalgesic response (Pecina et al. 2014).

A PET study in an animal model of placebo analgesia has confirmed that the intrinsic μOR system in the medial prefrontal cortex is involved in the placebo effect (Zeng et al. 2018).

When heat pain was combined with either acupuncture or placebo needle stimulation in healthy volunteers, acupuncture was shown to result in altered binding of [^{11}C]DPN (mainly decreases) in the orbitofrontal cortex, medial prefrontal cortex, insula, thalamus, and anterior cingulate cortex (Dougherty et al. 2008).

Opioid receptor availability at baseline conditions appears to be a predictor of human sensitivity to both painful (cold pressor test) and mechanical stimuli (touch, vibration). Greater sensitivity was associated with a lower BP of [^{18}F]DPN in various cortical areas (Mueller et al. 2010). In a later study on 12 healthy male volunteers, a significant correlation was observed between baseline binding potential of [^{11}C]CFN (μOR binding) in the striatum and the cold pressor pain threshold (Hagelberg et al. 2012).

A Japanese study examined opioid mechanisms underlying heterotopic nociceptive counter-stimulation (HCNS). HCNS aims to activate endogenous pain inhibition processes, which can be beneficial in pain treatment. The right sural nerve of eight healthy volunteers was transcutaneously stimulated, resulting in leg pain. Immersion of the left hand in cold water was applied as HCNS. A late component of somatosensory evoked potentials, reflecting activity of the anterior cingulate cortex, was reduced by HCNS, and this reduction was associated with a higher binding potential of [^{11}C]CFN in the right amygdala at baseline. Activation of μOR in the amygdala may thus be involved in the anti-nociceptive effects of HCNS (Piché et al. 2014).

Since peripheral inflammatory factors can influence central nervous system functioning, and, on the other hand, central mechanisms such as neural activity can modulate peripheral immune function, an American study examined relationships between IL-1 family cytokine levels in plasma and μOR availability in human brain. Thirty-four healthy volunteers (22 female, 12 male) were scanned with [^{11}C]CFN in the presence and absence of a standardized pain challenge (intramuscular infusion of hypertonic saline by a computer-controlled pump). Cytokine levels in plasma were measured during both conditions. Higher baseline levels of IL-1 β were shown to be associated with lower binding potentials of [^{11}C]CFN in the amygdala and greater sensitivity to pain. Subjects with a greater pain-induced increase in IL-1 β experienced less endogenous opioid analgesia. Activation of μOR s in ventral caudate and nucleus accumbens during the pain challenge was significantly associated with levels of IL-1 α , an anti-nociceptive cytokine. Thus, cytokine levels in human plasma and the endogenous opioid neurotransmitter system seem to interact (Prossin et al. 2015).

A PET study from Finland has indicated that vicarious pain (i.e., seeing others in pain) activates specific regions in the human brain and that μOR but not D_2R are involved in this response. Thirty-five healthy subjects were scanned, using the μOR tracer [^{11}C]CFN and the D_2R tracer [^{11}C]raclopride. Functional MRI scans of their brains were also made, during the watching of videoclips that depicted humans in painful or painless situations. The fMRI scans demonstrated increases of blood flow in various brain regions when the subjects watched painful scenes. In anterior and posterior insulae, thalamus, secondary and primary somatosensory cortices, primary motor cortex and superior temporal sulci, the magnitude of these increases was negatively correlated to measured [^{11}C]CFN binding potentials, whereas the response in orbitofrontal cortex showed a positive correlation to μOR availability. In contrast to μOR , dopamine D_2R availability was not correlated to the blood flow response in any brain region (Karjalainen et al. 2017). Thus, inter-individual differences in μOR availability may explain why some humans react more strongly than others to the seeing of pain.

21.4.7 Vestibular Processing

An interesting study examined involvement of the opioid system in vestibular neurotransmission in humans. Ten right-handed healthy volunteers were scanned with the ligand [^{18}F]DPN, both under baseline conditions and during caloric stimulation of the right ear. Each 90-min scan session consisted of three subsequent phases: a prestimulation period (30 min), a stimulation or sham period (30 min), and a follow-up period (30 min). During the stimulation period, warm (44 °C) and cold (30 °C) air were alternated at 5-min intervals in order to avoid habituation; during the sham period, the tubing was fixed to the subjects head in such a way that the air flow passed beside the ear. A decrease in tracer binding was noted in the right posterior insular cortex and the postcentral region during stimulation, indicating release of endogenous opioids and involvement of the opioid system in vestibular processing (Baier et al. 2010).

21.4.8 Myocardial Opioid Receptors

Both [^{11}C]N-methyl-naltrindole ([^{11}C]MeNTI) and [^{11}C]CFN show specific binding in the human heart. However, the reduction of myocardial distribution volume and binding potential of these opioid ligands after pretreatment of volunteers with naloxone is only minor (14–21 and 19–25%, respectively), suggesting low levels of δOR and μOR expression and poor signal-to-noise ratios in PET (Villemagne et al. 2002).

21.4.9 Occupancy Studies

PET offers the unique opportunity of measuring the fraction of receptor populations occupied by nonradioactive drugs in the living brain and relating measured levels of occupancy to the magnitude of the therapeutic effect or to unwanted side effects. Receptor occupancy is estimated by assessing competition of a nonradioactive drug with the radioligand for the same binding sites. The basic idea was already published in 1980 (Homcy et al. 1980).

Using the radioligand [^{11}C]CFN, a half-life of μOR blockade of 72–108 h was measured in the brain of healthy volunteers after a single oral dosing of naltrexone, corresponding to the duration of pharmacological effects when the drug is used for treatment of heroin dependence (Lee et al. 1988).

A later study with [^{11}C]CFN compared the duration of occupancy of μOR in the human brain by the antagonists nalmefene and naloxone. Clearance half-lives were 28.7 h after administration of 1 mg of nalmefene and 2.0 h for 2 mg of naloxone. The longer blockade by nalmefene could represent an advantage in the clinical use of this compound for reversal of opioid anesthesia or for treatment of drug addicts after an opioid overdose (Kim et al. 1997). Nalmefene has also been proposed for treatment of alcoholism. The decline of μOR occupancy in the human brain after single or repeated nalmefene dosing was found to be slower than the decline in

plasma concentration of the drug or its metabolites, indicating slow dissociation of receptor-bound nalmefene *in vivo* (Ingman et al. 2005).

Buprenorphine is used for the treatment of heroin dependence. Buprenorphine occupancy of μ OR in the human brain has been examined, using [^{11}C]CFN and PET. Four hours after administration of 2 mg buprenorphine, receptor occupancy ranged from 36 to 50%. When the buprenorphine dose was raised to 16 mg, levels of occupancy increased to 79–95%. Placebo-treated heroin abusers had greater μ OR binding potential in the inferofrontal and anterior cingulate cortex compared to matched healthy controls (Zubieta et al. 2000). Increasing doses of buprenorphine were associated both with decreased μ OR availability and with decreased withdrawal symptoms. The highest dose of buprenorphine (32 mg) caused virtually complete μ OR occupancy (Greenwald et al. 2003). The duration of action of buprenorphine was examined in ten heroin-dependent subjects after termination of a 16 mg/day dose of the drug. Relative to a control group of heroin-dependent subjects maintained on placebo, μ OR occupancy was 70, 46, 33, and 18% at 4, 28, 52, and 76 h after last use of the drug. Receptor occupancy was correlated with withdrawal symptoms; an occupancy of 50–60% appeared necessary for adequate symptom suppression (Greenwald et al. 2007).

Nasal spray formulations of naloxone may be useful in the treatment of victims of opioid overdose and may also be used for treating subjects with gambling disorder and alcohol addicts. Occupancy of the μ OR population in the human brain after intranasal spray dosing of naloxone has been assessed in healthy volunteers with [^{11}C]CFN and PET. Naloxone levels in the brain were found to peak within half an hour, a bit later than levels in plasma which peaked at 20 min. Receptor occupancies after intranasal administration of 2 and 4 mg naloxone were 67 and 85%, respectively, whereas the clearance half-life of the bound drug was about 100 min (Johansson et al. 2019).

The κ OR antagonist LY2456302 has been developed for treating neuropsychiatric disorders and substance abuse. Dose-dependent target occupancy by this drug has been measured in a PET study with [^{11}C]LY2795050 in 30 healthy volunteers. An oral dose of 10 mg resulted in virtually complete κ OR occupancy and was well-tolerated (Naganawa et al. 2016).

Although the dose- and time-dependent opioid receptor occupancy by antagonists and partial agonists can be assessed with PET, measurements of receptor occupancy by full agonists may be problematic. No difference in [^{11}C]DPN binding was found in the brain of opioid-dependent subjects stable on methadone (18–90 mg daily, PET scan performed at peak plasma levels of the agonist) as compared to healthy controls. In rats receiving increased doses of methadone, no dose-dependent reduction of the cerebral binding of [^{11}C]DPN was observed. These negative findings were explained by assuming that methadone is already efficacious at very low levels of receptor occupancy (<10%, i.e., below the detection limit of PET) (Melichar et al. 2005). In a subsequent preclinical study, rats were pretreated with antinociceptive doses of oxycodone (μ OR and κ OR agonist), morphine (μ OR agonist), and buprenorphine (δ OR and κ OR antagonist, μ OR partial agonist). Full agonists did not reduce the brain uptake of [^{11}C]DPN at all, whereas buprenorphine administration resulted in a strong reduction (up to 90%) of tracer binding (Hume et al. 2007).

Opioid receptor occupancy by the antagonist naloxone could also be measured with [^{11}C]DPN and PET, a drug dose of 13 $\mu\text{g/kg}$ resulting in 50% occupancy (Melichar et al. 2003). The reason for the negative findings with exogenous full agonists is not clear, since endogenous enkephalins have been shown to compete with [^{11}C]DPN for OR binding in the human brain (Ruiz-Gayo et al. 1992). Moreover, an early PET study reported 19–32% lower binding of [^{18}F]cyclofoxy in various brain regions of long-term methadone-treated former heroin addicts as compared to controls (22 h after the last dose), and the reduction of tracer binding in caudate and putamen correlated with levels of methadone in plasma (Kling et al. 2000). Since the observed competition is both tracer and agonist dependent, these PET data may indicate the presence of multiple, different agonist binding sites on opioid receptor proteins or upregulation of subclasses of opioid receptors in heroin-dependent subjects.

Opioid receptor (particularly μOR) agonists increase and antagonists decrease feeding and other rewarding behaviors in animal models. A [^{11}C]CFN-PET study examined cerebral μOR occupancy in 26 healthy male volunteers after single oral doses of either GSK1521498 (μOR inverse agonist, candidate drug for the treatment of overeating) or naltrexone (μOR and κOR antagonist). Activation of the amygdala by a palatable food stimulus (fruit drink) was examined in the same volunteers, using fMRI. Although a dose-dependent occupancy of μOR was observed for both compounds, GSK1521498 significantly attenuated activation of the amygdala by the food stimulus, in contrast to naltrexone. This difference may be related to the fact that μOR and κOR signaling have different effects on feeding behavior and reward processing (Rabiner et al. 2011).

Naloxone administration triggers activity of the hypothalamic-pituitary-adrenal (HPA) axis by blocking opioid inhibitory tone at corticotropin-releasing factor (CRF) neurons in the paraventricular nucleus of the hypothalamus. These neurons regulate the secretion of adrenocorticotrophic hormone (ACTH) and cortisol. An interesting study measured μOR binding potential in the brain of 18 healthy subjects using [^{11}C]CFN and PET. The following day, ACTH and cortisol responses to an incremental naloxone challenge were tested in the same subject group. Cortisol responses to naloxone were negatively correlated to [^{11}C]CFN binding potential in several brain areas (ventral striatum, putamen, caudate, hypothalamus). Apparently, subjects with higher binding potential have lower endogenous occupancy of μOR by β -endorphin and thus place less inhibitory tone on the HPA axis; therefore these subjects show a smaller cortisol response to naloxone (Wand et al. 2011).

21.5 PET Studies in Patients and Drug Addicts

21.5.1 Major Depressive, Borderline Personality, and Posttraumatic Stress Disorder

Using the sustained neutral and sadness state paradigm, μOR availability in the brain of healthy female volunteers and patients with major depressive disorder (MDD) has been compared. Healthy controls showed an increase of μOR

availability in the anterior cingulate during the sustained sadness condition indicating deactivation of μ OR-mediated neurotransmission, but in patients who did not respond to antidepressant treatment after PET imaging, sustained sadness was associated with a *decreased* binding of [^{11}C]CFN in this region, i.e., activation of the opioid system. Patients with MDD showed significantly lower μ OR binding potential in the posterior thalamus than healthy controls during the neutral state. These PET data indicated that endogenous opioid neurotransmission is altered in MDD (Kennedy et al. 2006). A later study from the same group showed that in MDD patients but not in healthy subjects, plasma levels of the inflammatory cytokine IL-18 are positively correlated with baseline μ OR binding potential and with activation of μ OR-mediated neurotransmission during a sadness challenge (Prossin et al. 2011). Thus, there may be a link between peripheral stress-activated proinflammatory mechanisms and central stress-activated neurotransmitter systems, such as the opioid system.

A study from the same group examined responses of the μ OR system to sustained sadness in patients with borderline personality disorder. During the baseline (neutral) state, patients showed greater [^{11}C]CFN binding than healthy controls in the bilateral orbitofrontal cortex, caudate, and nucleus accumbens besides the left amygdala but lower binding in the posterior thalamus. Induction of sadness caused greater declines of [^{11}C]CFN binding potential in the pregenual anterior cingulate, left orbitofrontal cortex, left ventricular pallidum, left amygdala, and left inferior prefrontal cortex of the patients than of healthy controls and greater increases of binding potential in the left nucleus accumbens, the hypothalamus, and the right hippocampus/parahippocampus. Apparently, the opioid system of patients with borderline personality disorder differs from healthy, age- and sex-matched control subjects both at baseline and in its response to a negative emotional challenge (Prossin et al. 2010). Altered opioid neurotransmission may also be a mechanism underlying modified limbic system activity and mood disorders in polycystic ovary syndrome (PCOS). Insulin-resistant women with PCOS showed a greater blood flow response in limbic regions of the brain to unpleasant images than healthy controls, and the extent of this limbic activation was positively correlated to μ OR availability in the right amygdala and left ventral anterior cingulate (Marsh et al. 2013).

An interesting study compared μ OR binding in the brain of 14 healthy male volunteers, 15 males with combat exposure but without posttraumatic stress disorder (PTSD), and 16 male patients with PTSD, using [^{11}C]CFN and PET. The two groups which had been exposed to war trauma showed lower μ OR binding in the amygdala, nucleus accumbens, dorsal frontal cortex, and insular cortex than healthy volunteers but higher μ OR binding in the orbitofrontal cortex. PTSD patients showed a significant reduction of μ OR binding in the anterior cingulate cortex compared to both other groups. Combat-exposed subjects without PTSD had lower μ OR binding in the amygdala but higher binding in the orbitofrontal cortex than either PTSD patients or healthy controls. Thus, the opioid system of the brain shows specific changes both after trauma and in PTSD (Liberzon et al. 2007).

Preclinical data have suggested that the dynorphin/ κ OR system may be related to symptoms of fear and depression after exposure to trauma. An American study used

the κ OR ligand [^{11}C]LY2795050 to scan the brains of 35 subjects with a wide range of exposure to trauma (ranging from healthy controls to subjects with PTSD, MDD, and generalized anxiety disorder). Measured distribution volumes of the tracer in amygdala, anterior cingulate cortex, and ventral striatum were significantly and negatively correlated with 24-h urinary cortisol levels and severity of loss (i.e., dysphoria, depressive symptoms, emotional numbing), but not with feelings of threat (i.e., fear) in the subject group (Pietrzak et al. 2014). In contrast to the findings of this initial study, a later pilot study with [^{11}C]GR103545 did not observe any significant difference of regional tracer distribution volume (i.e., κ OR availability) between 10 patients with MDD and 13 healthy volunteers (Miller et al. 2018).

If medication-free patients with MDD and healthy controls were scanned with [^{11}C]CFN and subjected to a social rejection and acceptance protocol (as described above, Sect. 21.4.3 (Hsu et al. 2013)), the patients showed a reduced release of opioids in various brain regions in response to rejection and a slower emotional recovery than the healthy subjects. During acceptance, only the healthy controls reported an increase in self-esteem and an increased desire for social interaction, which was correlated with release of endogenous opioids in the nucleus accumbens. A reduced activity of the endogenous opioid system may thus be involved in the pathophysiology of MDD, resulting in a less effective recovery from negative and a decreased pleasure from positive social interactions (Hsu et al. 2015).

Another PET study from the same group examined changes of the regional binding potential of [^{11}C]CFN and plasma levels of interleukin-18 in 28 human volunteers during the induction of sad or neutral mood, using a standard protocol. The subject group consisted of 15 healthy controls and 13 patients with unmedicated MDD. All subjects were female. IL-18 levels were increased during sadness, indicating peripheral immune activation and were reduced during neutral mood. IL-18 increases in the patients were significantly greater than in the control group. Moreover, these increases were found to be linearly proportional to sadness-induced μ OR activation (i.e., reductions of tracer binding potential) in left ventral pallidum, bilateral anterior cingulate cortices, right hypothalamus, and bilateral amygdala. Apparently, changes in mood regulate human immune function, and this regulation may be related to central opioid neurotransmission (Prossin et al. 2016).

An interesting study with [^{11}C]CFN examined the impact of placebo and antidepressant treatment in 35 medication-free patients with MDD. All subjects were treated with a placebo during two periods of 1 week. They were either informed that the placebo was a fast-acting antidepressant drug or that it was an inactive control. After each week of treatment, a [^{11}C]CFN-PET scan was made. In half of the subjects, the “active” placebo was given first and was followed by the “inactive” placebo, whereas in the other half, the order of treatments was reversed. After both PET scans and placebo treatments, all subjects were treated for 10 weeks with a real antidepressant, in most cases a selective serotonin reuptake inhibitor.

Higher μ OR binding in the nucleus accumbens at baseline was associated with better antidepressant response. A decrease of tracer binding potential in subgenual anterior cingulate cortex, nucleus accumbens, thalamus, and amygdala after 1 week of “active” placebo (compared to the amount of binding after 1 week of “inactive”

placebo) was associated with placebo-induced reductions in depressive symptoms and also with a better response to the real antidepressant at the end of the trial. Thus, a placebo is capable of activating the endogenous μ -opioid system in patients with MDD and this system is also involved in the response of such patients to an antidepressant drug (Pecina et al. 2015).

21.5.2 Pain

In a group of four patients with rheumatoid arthritis, regional cerebral opioid receptor binding of [^{11}C]DPN was quantified both during a period of pain and during a period when the subjects were out of pain. Significant decreases of [^{11}C]DPN binding were seen in many brain areas when subjects suffered pain (particularly straight gyrus and frontal, cingulate, and temporal cortex) (Jones et al. 1994b). In a patient with central poststroke pain which developed after a small pontine hemorrhagic infarction, a reduction of the binding of [^{11}C]DPN was also noted, and this reduction was more striking than the hypometabolism of [^{18}F]FDG on the lateral cortical surface contralateral to the symptoms (Willoch et al. 1999).

Changes of [^{11}C]DPN binding in the brain of six patients after surgical relief of trigeminal neuralgia pain were examined in a later study. The volume of distribution of the tracer in several cortical areas (prefrontal, insular, perigenual, midcingulate, inferior parietal), basal ganglia, and the thalamus was found to be significantly increased after surgery (Jones et al. 1999). These data and the findings in the previous studies were interpreted as evidence for the release of opioid peptides during various forms of pain resulting in a reduction of the fraction of the receptor population available for ligand binding. However, changes of opioid receptor numbers (upregulation after surgery or after the disappearance of pain) could not be ruled out.

In a later study involving four patients with central neuropathic pain (mainly poststroke) and age-matched controls, patients were found to have significantly less [^{11}C]DPN binding in the dorsolateral, anterior cingulate, and insula cortices, the thalamus, and the inferior parietal cortex. These reductions were not a direct consequence of their cerebral lesions, since these were located in other brain areas. The authors interpreted the PET data as evidence for reduced damping of nociceptor activity in the patients and as a possible explanation for the fact that high doses of opiates are frequently required to achieve optimal analgesia in subjects with central neuropathic pain (Jones et al. 2004). Essentially similar findings were reported in a study from another institution, using the same tracer and involving 5 patients with central poststroke pain and 12 healthy volunteers (Willoch et al. 2004).

An interesting study compared changes of [^{11}C]DPN binding in the brain of patients with peripheral neuropathic pain and central poststroke pain. Peripheral neuropathic pain patients showed bilateral and symmetric decreases of [^{11}C]DPN binding, in contrast to central poststroke pain patients where an asymmetric decrease was noted, which was most prominent in the contralateral hemisphere. The symmetric and bilateral decline in the former patient group may reflect endogenous opioid release, whereas the lateralized decrease in the latter group suggests a loss of

opioid receptors (Maarrawi et al. 2007a). Thus, central and peripheral forms of neuropathic pain may have different effects on the opioid system of the brain.

In a group of ten patients with restless legs syndrome (RLS), significant negative correlations were observed between [^{11}C]DPN binding in areas serving the medial pain system and RLS severity or pain scores. These findings suggested release of endogenous opioids within the medial pain system related to the severity of the syndrome (von Spiczak et al. 2005).

Changes of the opioid system in patients with cluster headache were also examined, using the tracer [^{11}C]DPN and PET. When patients as a group were compared with healthy volunteers, a striking (>50%) decrease of tracer binding was noted in the pineal gland but not in any other brain area. Within the patient group, opioid receptor availability in the ipsilateral hypothalamus and anterior cingulate cortex was negatively correlated to the duration of the headache disorder. The patients did not experience acute pain during or immediately before the study; thus, the observed alterations of [^{11}C]DPN binding appear to not represent an effect of acute pain processing but rather alterations in the opioid system related to the development of the disease. Opioidergic dysfunction in circuitries generating the biologic clock may be a mechanism underlying cluster headache (Sprenger et al. 2006b).

In patients with complex regional pain syndrome (CRPS), a chronic pain condition which can develop after limb trauma, binding of the non-subtype-selective OR ligand [^{18}F]DPN was found to be reduced in the contralateral amygdala and parahippocampal gyri but increased in the contralateral prefrontal cortical areas. Ligand binding in the ipsilateral temporal cortex and midcingulate cortex was negatively correlated with pain scores, but ligand binding in the contralateral temporal cortex was positively correlated with anxiety and depression scales (Klega et al. 2010). These findings suggest that the endogenous opioid system is implied in both chronic pain and its psychiatric comorbidity.

A study with the tracer [^{11}C]CFN compared μOR binding in the brain of four patients with trigeminal neuropathic pain and eight gender- and age-matched healthy controls. Patients showed reduced tracer binding in the left nucleus accumbens, and binding potential in this area was negatively correlated with their pain ratings. This finding was interpreted as evidence for downregulation of μOR after persistent activation of opioid neurotransmission in chronic pain (DosSantos et al. 2012a). A recent PET study in experimental animals has proven that reduced μOR availability in chronic neuropathic pain (measured with [^{18}F]DPN and PET) reflects reduced μOR expression. In this study, μOR expression in various brain areas was quantified by ex vivo immunohistochemistry (Thompson et al. 2018).

Another PET study with [^{11}C]CFN examined regional μOR binding potential values in the human brain during experimentally induced pain (application of 0.35 g of 10% capsaicin cream on a 6.25 cm² area of the left hand). The data from the PET study were compared to evaluations of the sleep quality of the subjects ($n = 14$) during the previous month, using the Pittsburgh Sleep Quality Index. Poor sleep quality was significantly and positively correlated to greater μOR binding potential in the frontal lobes, and sleep duration was negatively associated with binding potential in these lobes, the temporal lobe and the anterior cingulate. Thus, individual variation

in the quality and duration of sleep appear to be associated with variation in cerebral μ OR binding during tonic pain. Poor sleep may be related to impaired pain-suppressing mechanisms (less release of endogenous opioids in response to a painful stimulus) and an increased perception of pain (Campbell et al. 2013).

A more complicated study with [^{11}C]CFN provided evidence for the hypothesis that both alterations in μ OR availability and a reduced release of endogenous opioids contribute to the clinical symptoms of patients suffering from chronic back pain. Tracer binding potential was measured in 16 patients with chronic back pain and 16 age- and gender-matched healthy volunteers under three different conditions: at baseline, during pain expectation (intramuscular injection of isotonic saline), and during sustained moderate pain (intramuscular injection of hypertonic saline). Baseline μ OR binding in the thalamus proved to be higher in the patients than in the control subjects, and the patients showed smaller reductions in μ OR binding in several brain areas, both during the painful condition and the expectation of pain (Martikainen et al. 2013).

PET with the μ OR tracer [^{11}C]CFN has also been used to examine activity of the opioid system during migraine attacks. Seven patients with frequent migraine were scanned both during the ictal and interictal phase. μ OR binding in the ipsilateral medial prefrontal cortex was found to be significantly reduced during the ictal phase, possibly due to increased release of endogenous opioids, in order to fight the ongoing pain. The magnitude of this reduction was negatively correlated to the combined extension and severity of the migraine attack (Dasilva et al. 2014).

Migraine, particularly in patients that have used opioids to suppress their pain, is frequently associated with allodynia, i.e., an increased sensitivity of the skin to stimuli that should not cause pain. Normal activities, such as washing the skin with warm water, or combing the hair, are painful for subjects suffering from allodynia. A report following on the previous PET study examined changes of [^{11}C]CFN in patient's brains during migraine-associated allodynia. The skin was thermally challenged by a device that provided heat pulses every 10s during a period of 20 min. Patients were challenged and scanned both during the ictal and interictal phase of their migraine. A decrease of μ OR binding potential was observed in the ventrolateral periaqueductal gray matter and the red nucleus area of the midbrain, when patients were thermally challenged and showed allodynia. The magnitude of this decrease was positively correlated to allodynic severity during a migraine attack (Nascimento et al. 2014).

Simultaneous PET (with the opioid receptor ligand [^{11}C]DPN) and functional MRI (fMRI) has been used to examine the opioidergic pain system in the human brain. Eight healthy volunteers were scanned twice on the same day, during the application of a painful and a non-painful stimulus (pressure). The order of the scans was randomized. Pain-related activation of several brain regions was observed in fMRI, but in most of these regions, opioid receptor binding was not significantly altered. Co-localized fMRI and PET signal changes were noticed only in the ipsilateral thalamus and the contralateral striatum. Decreases of tracer binding potential and increases of blood flow (fMRI BOLD signal) were significantly correlated in the thalamus, suggesting that in this area of the brain, enhanced opioid

neurotransmission is a major cause of the increases of flow, i.e., of neuronal activation. However, no correlation was observed between the PET and fMRI signal changes in the striatum, suggesting that in this region, additional receptor systems were contributing to the observed flow changes (Wey et al. 2014).

A British study used the PET tracer [^{11}C]DPN to relate opioid receptor availability in the human brain to pain perception in arthritis. Within the patient group with arthritis ($n = 17$), the perception of higher levels of chronic pain in the week before the scan was associated with higher OR availability, particularly in the caudate, nucleus accumbens, and subcallosal area. This observation suggests that opioid receptors are upregulated in individuals experiencing greater chronic pain. Higher OR availability was associated with higher acute thermal pain thresholds, suggesting that OR upregulation is an adaptive response aimed at dampening the pain perception. When the entire patient group was considered, OR availability in the patients caudate nucleus was reduced in comparison to healthy controls ($n = 9$), suggesting a greater release of endogenous opioid ligands and greater occupancy of ORs in the arthritis group (Brown et al. 2015).

Patients with fibromyalgia report widespread pain in muscles and connective tissue, whereas no pathology can be identified in the painful body areas. The central nervous system of these patients may have become overly sensitive to painful stimuli. In order to test this hypothesis, pain-evoked increases of cerebral blood flow were quantified with fMRI and regional μOR availability was quantified in 18 female patients with fibromyalgia, using PET and [^{11}C]CFN. Patients showed a lower μOR availability in dorsolateral prefrontal and anterior cingulate cortex than healthy controls, and this was associated with a lower blood flow response to painful stimuli. Thus, the endogenous opioid system appears to be dysregulated in fibromyalgia, resulting in lower activation of antinoceptive brain regions after incoming painful stimuli and abnormal sensitivity to pain (Schrepf et al. 2016).

21.5.3 Pain Treatment

Monitoring the relationship between opioid receptor occupancy and pain relief could be useful in the development of improved techniques for the treatment of pain (Sadzot et al. 1990; Sadzot and Frost 1990). This study paradigm has been employed in several reports.

An initial study examined the contribution of the endogenous opioid system to pain relief induced by motor cortex stimulation (MCS). Eight patients with refractory neuropathic pain were scanned three times with [^{11}C]DPN and PET: twice preoperatively with a 2-week interval and once after 7 months of chronic MCS. The preoperative test-retest scans did not show any significant differences, but comparison of the last scan with the two preoperative scans revealed significant decreases of tracer binding in the anterior middle cingulate cortex, periaqueductal gray, prefrontal cortex, and cerebellum. The magnitude of the changes in the first two brain areas was significantly correlated with pain relief. These findings were interpreted as

evidence for the release of endogenous opioids in brain structures involved in pain processing after MCS (Maarrawi et al. 2007b).

The effects of traditional Chinese acupuncture and placebo needle stimulation have been compared in chronic pain patients diagnosed with fibromyalgia. [^{11}C]CFN-PET scans were made at both the beginning and the end of the 4-week treatment period. Acupuncture was shown to result in both short- and long-term increases in [^{11}C]CFN binding potential in multiple pain and sensory processing regions. In the sham group, small reductions of [^{11}C]CFN binding were observed, consistent with previously reported placebo effects. The long-term increases after acupuncture were correlated with reductions in clinical pain. Thus, acupuncture and sham acupuncture may have different effects on cerebral μOR binding and alterations of μOR binding potential may be involved in the analgesic effect of acupuncture (Harris et al. 2009).

A case study reported that transcranial direct current stimulation (tDCS) of the motor cortex in a trigeminal neuropathic pain patient considerably decreased the binding potential of [^{11}C]CFN in several brain areas compared to sham tDCS. The effect was particularly striking in the posterior thalamus. tDCS increased the threshold for experimental cold pain in the patient but did not improve her clinical pain. The authors suggested that the release of endogenous opioids is immediately increased by tDCS, but repetitive tDCS sessions are required to revert neuroplastic changes related to the neuropathic pain (DosSantos et al. 2012b). A follow-up study from the same group examined the mechanisms underlying tDCS. Nine healthy volunteers were scanned with [^{11}C]CFN on two different occasions: at baseline and during application of tDCS. The last scan comprised a placebo phase (in which tDCS electrodes were present but no stimulation was applied) and a real stimulation phase. The placebo phase was associated with a decrease of tracer binding potential in the periaqueductal gray, precuneus, and thalamus, indicating release of endogenous opioids in response to the placebo. Real tDCS was associated with similar changes in periaqueductal gray and precuneus and an additional decrease of tracer binding in the left prefrontal cortex. Significant analgesic effects (increases of pain thresholds) were only observed after real tDCS. Thus, the endogenous μOR system is already activated by a placebo, and the placebo effect seems to be extended and optimized by real tDCS (DosSantos et al. 2014).

A PET study with [^{11}C]DPN examined OR availability in 15 patients suffering from refractory neuropathic pain. The patients were subsequently treated by receiving chronically implanted motor cortex stimulation. Preoperative levels of opioid binding in several brain regions (insula, thalamus, periaqueductal grey, anterior cingulate, and orbitofrontal cortex) were found to be significantly and positively correlated with postoperative pain relief after 7 months. Thus, PET scans with an opioid receptor ligand may help clinicians to select patients with neuropathic pain, who are likely to benefit from motor cortex stimulation (Maarrawi et al. 2013).

A Chinese study evaluated the impact of 30 min of transcutaneous electrical acupoint stimulation (TEAS) on μOR availability in the brain of anaesthetized rhesus monkeys, using [^{11}C]CFN and PET. The study showed that 2 Hz but not 100 Hz stimulation resulted in a significant increase of tracer binding potential in the

anterior cingulate cortex, caudate nucleus, putamen, temporal lobe, somatosensory cortex, and amygdala (compared to a 0 Hz control scan). These results suggest that not only the release of endogenous opioid peptides, but also an upregulation of μ OR plays a role in the mechanism of action of TEAS (Xiang et al. 2014).

A decreased availability of μ OR in the human brain (suggesting the release of endogenous opioids) has also been observed in healthy volunteers ($n = 10$) after transcranial magnetic stimulation (TMS). After active but not sham TMS, the binding potential values of [11 C]CFN were lowered in the right ventral striatum, medial orbitofrontal, prefrontal and anterior cingulate cortices, left insula, superior temporal gyrus, dorsolateral prefrontal cortex, and precentral gyrus. In contrast to these changes of μ OR, dopamine D2R availability was not altered after TMS (Lamusuo et al. 2017).

21.5.4 Substance Abuse

21.5.4.1 Cocaine Dependence

The endogenous opioid system appears to play a role in the reinforcing actions of non-opioid drugs. American investigators examined μ OR binding in the brain of cocaine addicts, 1–4 days after their last use of cocaine, using [11 C]CFN and PET. An increase of tracer binding was observed, particularly in the frontal cortex and caudate nucleus, and this increase persisted even after 4 weeks of drug abstinence. The magnitude of [11 C]CFN uptake was positively correlated with the severity of cocaine craving experienced by the subjects. These findings confirmed the involvement of the opioid system in cocaine dependence (Zubieta et al. 1996).

Increases of μ OR binding potential in the anterior cingulate and anterior frontal cortex of cocaine users during abstinence are not only positively correlated with the intensity of cocaine craving, but the elevation of [11 C]CFN binding in these areas is also correlated with the percentage of days with cocaine use and the amount of cocaine used per day before the study period (Gorelick et al. 2005).

Regional increases of μ OR binding appear to be related not only to the intensity of cocaine craving but also to the time interval before relapse to cocaine use after discharge from a closed research ward. In an interesting study, 15 cocaine-dependent subjects were housed in this ward for 12 weeks of monitored abstinence. During this period, regional brain μ OR binding was measured at 1 and 12 weeks, using [11 C]CFN and PET. A shorter time interval before relapse was associated with increased μ OR binding in the frontal and temporal cortex in both PET scans and with a lesser decrease in binding between 1 and 12 weeks. The significance of this correlation persisted even after accounting for clinical variables. Thus, increased μ OR binding in the frontal and temporal cortex is a predictor of time to relapse to cocaine use (Gorelick et al. 2008).

A PET study examined regional μ OR ([11 C]CFN) binding in the brain of treatment-seeking cocaine users before the onset of treatment and correlated this parameter with subsequent treatment outcome. Elevated μ OR binding in brain regions associated with reward sensitivity correlated with greater cocaine use

during treatment and shorter duration of cocaine abstinence. Thus, μ OR binding in these brain areas was a significant independent predictor of treatment outcome (Ghitza et al. 2010).

The mechanism of action of psychostimulant drugs in the mammalian brain appears to involve the release of endogenous opioids (endorphins) in the reward system. Two recent PET studies have attempted to obtain experimental proof for this mechanism by administering d-amphetamine to healthy volunteers (i.v.) and examining the impact of this challenge on [^{11}C]CFN binding in the brain. A Swedish study in which ten subjects were scanned under three conditions (baseline, placebo, amphetamine 0.3 mg/kg) could not detect any significant differences in the binding potential of [^{11}C]CFN after the amphetamine challenge (Guterstam et al. 2013). However, another study in which 12 subjects were scanned before and 3 h after an amphetamine challenge (either a high dose, 0.5 mg/kg, or an ultralow dose 0.017 mg/kg) reported that [^{11}C]CFN binding was reduced after the high but not the ultralow dose in the frontal cortex, putamen, caudate, thalamus, anterior cingulate cortex, and insula (Colasanti et al. 2012).

21.5.4.2 Opioid Dependence

Similar changes of opioid receptor binding as were reported for cocaine addicts have also been observed in early abstinence from opioid (heroin) dependence. Compared with 20 healthy controls, ten subjects with opioid dependence who had completed inpatient detoxification showed increased [^{11}C]DPN binding in the whole brain and in 15 out of 21 studied brain areas (Williams et al. 2007).

21.5.4.3 Alcohol Dependence

Whereas studies in cocaine and heroin addicts suggest increases of opioid receptor binding during abstinence, studies in alcohol dependence have produced conflicting results. In an early PET study with [^{11}C]CFN, μ OR binding potential was measured in eight male alcohol-dependent subjects during alcohol withdrawal and eight matched healthy controls. Contrary to the findings in cocaine abusers, alcoholics showed *reductions* of μ OR binding potential in the right dorsal lateral prefrontal cortex, right anterior frontal cortex, and right parietal cortex. Lower μ OR binding potential was associated with higher craving and with depressive symptoms in the subjects (Bencherif et al. 2004b). Based on the findings in subsequent studies, one could speculate that alcohol abstinence and depression have different effects on μ OR binding (an increase and a decrease, respectively), the latter effect predominating in this early investigation.

Opposite results were reported in a German study. The authors examined [^{11}C]CFN binding in the brain of alcoholics after 1–3 weeks of abstinence. Significantly *higher* binding was observed in the ventral striatum of the patients compared with healthy controls. This increase persisted up to 5 weeks, and its magnitude was significantly correlated with the intensity of alcohol craving. Elevated availability of μ OR in the striatum was considered as a neuronal correlate of alcohol urge since the ventral striatum is known to be an important part of the reward system in the human brain (Heinz et al. 2005). Similar results were reported in a large study

involving 25 alcohol-dependent subjects who were scanned on day 5 of abstinence and 30 healthy controls. Alcohol-dependent subjects had a significantly higher binding potential of [^{11}C]CFN in many brain regions. There was a significant inverse relationship between [^{11}C]CFN binding in several brain regions and the intensity of craving in the alcohol-dependent group. No significant group differences in binding of the δOR ligand [^{11}C]MeNTI were observed, but the binding potential of this ligand in the caudate nucleus was positively correlated with recent drinking in alcohol-dependent subjects (Weerts et al. 2011).

In alcohol-dependent subjects completing a 19-day inpatient protocol (alcohol abstinence followed by naltrexone treatment, 50 mg daily, on days 15–19), a virtually complete (95%) occupancy of μOR by naltrexone was observed, using [^{11}C]CFN and PET. However, a much lower and highly variable (0–50%) occupancy of δOR was detected in [^{11}C]MeNTI scans. The relationship between δOR occupancy and treatment outcome could be explored in future studies (Weerts et al. 2008).

A British study with the non-subtype-selective opioid ligand [^{11}C]DPN reported a trend toward increased tracer binding in 11 alcohol-dependent subjects during early abstinence, compared to healthy controls, although this trend did not reach statistical significance. A significant correlation between alcohol craving and the global and regional distribution volume of [^{11}C]DPN was noted in the alcohol-dependent subjects (Williams et al. 2009).

A recent study examined [^{11}C]CFN binding in the human brain before and immediately after alcohol consumption in 13 heavy drinkers and an age-matched control group of 12 healthy subjects. Drinking alcohol reduced tracer binding in the nucleus accumbens and orbitofrontal cortex, indicating release of endogenous opioids in brain areas involved in the valuation of reward. The magnitude of change in the orbitofrontal cortex was significantly correlated with problem alcohol use and with subjective high in the drinker group. Thus, the release of endogenous opioids by ethanol promotes further alcohol consumption, and altered function of the opioid system appears to contribute to alcohol abuse (Mitchell et al. 2012).

Increases of the plasma level of the hormone cortisol after administration of an opioid antagonist (such as the drug naloxone) are supposed to provide information about opioid receptor activity. In an American study, naloxone was administered to 18 healthy control and 25 alcohol-dependent subjects (after 5 days of medically supervised alcohol withdrawal). The dose of naloxone required to induce a peak cortisol response was two times higher in the alcohol-dependent group. PET scans with [^{11}C]CFN were made in all subjects, and the relationship between tracer binding potential and rising slope of the cortisol response was examined. Alcohol-dependent subjects showed a significantly higher tracer binding potential in several brain regions than healthy controls, as was previously reported (Weerts et al. 2011). In healthy controls, there was a significant negative correlation between cortisol slope and regional binding potentials of the tracer, but in alcohol-dependent subjects, these parameters were not correlated. Thus, in healthy subjects, the naloxone test can provide information about individual differences in μOR availability, but in shortly abstinent alcohol-dependent subjects, the relationship between naloxone-induced cortisol response and μOR availability is disrupted (Wand et al. 2012).

In a follow-up study from the same group, PET scans with the δ OR ligand [^{11}C]methylnantrindole were made in 15 healthy control and 20 shortly abstinent alcohol-dependent subjects, and δ OR binding potential in various regions of the brain was correlated to cortisol area under the curve (AUC) after administration of naloxone. Similar to the findings for μ OR, significant negative correlations were observed between cortisol AUC and δ OR binding potential in several brain regions of healthy subjects. However, in alcohol-dependent subjects, such correlations were not observed. Thus, in healthy subjects, the naloxone test provides information about individual differences in δ OR availability, but in alcohol-dependent subjects, the close relationship between opioidergic neurotransmission and hormonal response is lost (Wand et al. 2013).

A PET study with the μ OR tracer [^{11}C]CFN examined the relationship between opioid release in the human brain after alcohol consumption and the catechol-O-methyltransferase genotype. Thirteen heavy drinkers and 12 matched healthy control subjects were scanned twice with [^{11}C]CFN: first at baseline and then after the consumption of a standard drink of alcohol. Independent of their drinking history (or gender), individuals with the Val158 variant of the enzyme showed greater opioid release in the right nucleus accumbens and less release in medial orbital frontal cortex. The Val158 variant results in greater activity of catecholamine-O-methyltransferase in the human brain and, consequently, lower dopamine levels in the frontal cortex. Apparently, genetic differences in endogenous dopamine levels can modulate endogenous opioid release in brain regions that contribute to the rewarding effects of alcohol. The Val158 variant could make individuals more prone to impulsive decision-making (Mitchell et al. 2013).

The higher binding potential values of [^{11}C]CFN in the brain of alcohol-dependent subjects that have been reported by various research groups may either reflect increased expression of μ OR, or a reduced concentration of endogenous, competing opioid ligands. In order to differentiate between these possible explanations, a German study examined post-mortem brain tissue of 43 alcohol-dependent subjects and 43 healthy controls. The density of μ OR in tissue samples was determined by *in vitro* assays, using the ligand [^3H]DAMGO. Tracer binding in the brain of alcohol-dependent subjects was 23–51% lower than in healthy controls. Thus, increased binding potential values of [^{11}C]CFN in the brains of alcoholics seem to reflect reduced competition of endogenous opioids rather than increased receptor expression (Hermann et al. 2017).

Addiction (to alcohol or other substances of abuse) may be considered as a state of “reward deficiency.” Addicts take a rewarding substance to compensate for their deficient reward. If this interpretation of addiction is correct, drug addicts should show a reduced release of endogenous opioids after a release-inducing stimulus compared to healthy controls. In a British PET study, 13 abstinent alcohol-dependent male subjects and 15 healthy volunteers were scanned twice with [^{11}C]CFN, before and 3 h after an oral dose of dexamphetamine. Consistent with the hypothesis, the healthy volunteers showed a significant decline of tracer binding potential in many areas of the brain after the amphetamine challenge, but in alcohol-dependent subjects, this response was reduced, particularly in frontal lobe, insula, thalamus,

anterior cingulate, nucleus accumbens, and putamen (Turton et al. 2018). In pathological gambling, a behavioral addiction with similarities to substance abuse, a similar “blunted” response of the opioid system to amphetamine was noted, although baseline μ OR availability was not different in pathological gamblers and healthy volunteers (Mick et al. 2016).

In an extensive PET study with the novel PET tracer [^{11}C]LY2795050, κ OR availability was measured in the brain of 36 alcohol-dependent subjects and 28 healthy controls. Tracer distribution volume in the amygdala and pallidum was significantly lower in the alcohol-dependent group than in the healthy control group. An age effect on tracer binding could not be observed in any brain region (Vijay et al. 2018). These observations for κ OR are in contrast with findings for μ OR or total OR that have been reported to be either increased or unchanged in alcohol-dependent subjects. Also, human μ OR show an age-related decline, whereas κ OR appear to be preserved in the aging human brain.

21.5.4.4 Nicotine Dependence

Several PET studies have examined the relationship between opioid neurotransmission and smoking. In an initial, groundbreaking publication, six healthy male smokers and six age- and sex-matched nonsmokers were scanned with both [^{11}C]CFN and the dopamine receptor ligand [^{11}C]raclopride. Smokers abstained from smoking for 12 h prior to the study. They first smoked a denicotinized cigarette, 2 and 12 min after scan onset, followed by a normal cigarette after 40 and 50 min. Total scan duration was 90 min. Thus, each scan period consisted of an initial, denicotinized phase and a later, average nicotine phase. Nonsmokers were subjected to the same scanning protocol but were only scanned at baseline (i.e., they did not smoke anything). In the smoker group, significant declines of [^{11}C]CFN binding in the right anterior cingulate cortex were noted during transition from the first to the second phase of the scan, indicating activation of opioid neurotransmission by nicotine. A simultaneous decline of [^{11}C]raclopride binding was noted in the ventral basal ganglia, and the magnitude of this decline correlated with nicotine dependence score. Increases of the release of endogenous opioids and dopamine are probably involved in the rewarding effect of nicotine. Smokers had lower μ OR binding potential in the cingulate cortex, thalamus, ventral basal ganglia, and amygdala compared to nonsmokers during the first phase of the scan; these reductions were reversed in the thalamus, ventral basal ganglia, and amygdala after nicotine smoking (Scott et al. 2007b). Greater μ OR availability in the basal ganglia and thalamus of nonsmokers compared to overnight abstinent smokers was also observed in a later study (Nuechterlein et al. 2016).

In contrast to the observations of Scott and Nuechterlein et al., another PET study with [^{11}C]CFN did not observe any significant change in tracer binding potential between placebo and active cigarette smoking and also no significant difference in μ OR availability between smokers and nonsmokers (Kuwabara et al. 2014). The discrepancy between this and the initial study led to the hypothesis that the expectancy of a smoker, or the sensorimotor effects of denicotinized cigarettes may contribute to the observed release of endogenous opioids. However, a negative

correlation was observed between μ OR availability in bilateral superior temporal cortices during placebo smoking and scores for nicotine dependence. Thus, μ OR in this area may play a role in nicotine addiction, and stimulation of these receptors by endogenous opioids may be involved in nicotine reward (Kuwabara et al. 2014).

Two recent PET studies showed that psychological and behavioral factors can indeed exert important actions on the μ -opioid system. In the first study, smokers showed a reduction of [^{11}C]CFN binding potential in the thalamus and nucleus accumbens after smoking a denicotinized (placebo) cigarette, indicating μ OR activation. No further activation was observed after the smoking of a normal cigarette (Nuechterlein et al. 2016). In the last study, 20 chronic tobacco smokers (males) were asked to abstain for one night from smoking. The next morning, they first smoked a denicotinized cigarette (at 8.00 h) and, subsequently, a normal cigarette with an average dose of nicotine (at 10.00 h). PET scans with [^{11}C]CFN were made either during the placebo or the nicotine smoking session (on two different days), using a bolus plus infusion protocol. The very low nicotine peak levels in plasma after smoking a denicotinized cigarette were significantly correlated with tracer binding potential after the smoking session, but no correlation was observed between the high peak levels after smoking of the normal cigarette and tracer binding potential (Domino and Hirasawa-Fujita 2019). Thus, “placebo” effects seem to have a major impact on [^{11}C]CFN binding in the smoker’s brain.

Another study with [^{11}C]CFN examined the relationship between smoking and the relief of negative affect. Twenty-two smokers were scanned after overnight abstinence, once after smoking a denicotinized cigarette and at another scanning day after smoking a normal cigarette. Higher μ OR availability in the amygdala was correlated with greater motivation to smoke in order to relieve negative affect, but not with changes in affect after smoking (Falcone et al. 2012). A positive correlation between the craving to smoke and μ OR binding potential in several brain regions (amygdala, hippocampus, insula, nucleus accumbens, putamen, and ventral striatum) was also reported in a later study (Domino and Hirasawa-Fujita 2019).

Polymorphisms of the μ OR gene affect both the levels of μ OR expression in the human brain and smoking behavior. Smokers homozygous for the wild-type *OPRM1* A allele show significantly higher [^{11}C]CFN binding in the bilateral amygdala, left thalamus, and left anterior cingulate cortex than smokers carrying the *OPRM1* A118G allele. In bearers of the G polymorphism, the extent of reward difference between smoking a normal and a denicotinized cigarette was significantly associated with the change of [^{11}C]CFN binding potential in the right amygdala, caudate, anterior cingulate cortex, and thalamus, but in smokers homozygous for the wild-type gene, this association could not be observed (Ray et al. 2011). Later PET studies in μ OR-genotyped overnight abstinent smokers confirmed that smokers bearing the G polymorphism have less free μ ORs in some brain areas (amygdala and nucleus accumbens) than carriers of the AA wildtype (Nuechterlein et al. 2016; Domino et al. 2015). In one of these studies, GG carriers were found to show also a less extensive decrease of [^{11}C]CFN binding potential after the smoking of a “real” cigarette than AA carriers, i.e., a blunted response of the μ -opioid system (Domino et al. 2015).

Another PET study examined whether individual differences in μ OR availability in alcohol-dependent subjects are associated with tobacco use, nicotine dependence, and level of nicotine craving. Subjects had withdrawn from alcohol use under medical supervision. They were not allowed to smoke but received transdermal nicotine maintenance (21 mg/day). Higher scores in a nicotine dependence test were found to be associated with a lower binding potential of [^{11}C]CFN in the amygdala, cingulate, globus pallidus, thalamus, and insula. The number of cigarettes which subjects had used per day prior to the study was also negatively correlated with [^{11}C]CFN binding potential in these areas. Thus, smoking intensity and severity of nicotine dependence appear to be related to reduced μ OR binding potential in several brain regions of alcohol-dependent subjects (Weerts et al. 2014).

21.5.5 Eating Disorders

Bulimia nervosa, a disorder characterized by cycles of food restriction, binge eating, and vomiting, shares certain phenomena with addiction and substance abuse. Compared with controls, bulimic individuals show significantly decreased μ OR binding in the left insular cortex, a brain area involved in taste discrimination and eating reward. This finding may reflect either downregulation of μ OR in the bulimic state as a consequence of chronically increased release of opioid peptides or a personality trait that increases the reward value of dieting (Bencherif et al. 2005).

In another PET study, 7 subjects with binge eating disorder, 15 subjects with pathological gambling, and 17 healthy control subjects were scanned with [^{11}C]CFN and [^{18}F]DOPA. Patients with binge eating disorder showed reductions of μ OR binding potential in many cortical and subcortical areas of the brain and a significant decrease of the striatal influx constant of [^{18}F]DOPA. However, in subjects with pathological gambling, μ OR binding potential was decreased mainly in the anterior cingulate, and the striatal influx of [^{18}F]DOPA was not significantly altered compared to healthy controls. Thus, two forms of addiction (to eating and to gambling) displayed different patterns of neurobiological changes (Majuri et al. 2017). A later study from the same group used [^{11}C]CFN to measure μ OR binding potential in the brains of patients with binge eating disorder, patients with morbid obesity and healthy controls. Both eating disorders were associated with similar, widespread reductions of μ OR binding potential. Thus, these two eating disorders shared a common opioid abnormality (Joutsa et al. 2018). In a third study from the same group, 13 pathological gamblers and 15 age-, sex-, and weight-matched healthy control subjects were scanned with [^{11}C]CFN and [^{18}F]DOPA. In both groups, a similar correlation was observed between μ OR availability in the basal ganglia (putamen, caudate nucleus and globus pallidus) and the presynaptic capacity for dopamine synthesis. Thus, presynaptic dopamine neurotransmission and opioid receptor function in the basal ganglia appear to be linked and this link is not affected by behavioral addiction (Majuri et al. 2018).

21.5.6 Obesity

An initial PET study on the involvement of endogenous opioid systems in obesity scanned seven chronically obese men with [^{11}C]CFN in the fasted and fed condition, at the onset of therapy and after 15% weight loss had been achieved by a low-calorie diet. Seven age- and ethnicity-matched lean control subjects were scanned only once, in the fasted and fed condition. Thus, four PET scans were made in the obese subjects and two scans in the healthy controls. In the initial scans, obese subjects showed lower μOR binding potentials in several brain regions than lean subjects or obese subjects after weight loss. In healthy controls, the observed decline of tracer binding potential in the right temporal pole after feeding was highly correlated to reductions in negative affect, but this correlation was not observed in the patient group. In patients, the magnitude of this decline at the onset of treatment tended to be associated with weight regain, 1 year after treatment. Thus, the magnitude of μOR activation in the right temporal pole after an acute meal may predict the risk of weight regain of obese subjects after therapy (Burghardt et al. 2015).

Since both the endogenous opioid system and the dopaminergic system are involved in the regulation of food intake and reward processing, a Finnish study examined pathological changes in μOR and dopamine D_2R availability in obese subjects. Thirteen obese (mean BMI 42 kg/m²) and 14 age-matched healthy women were scanned with the PET tracers [^{11}C]CFN and [^{11}C]raclopride. Obese subjects had significantly lower μOR binding potentials in ventral striatum, orbitofrontal cortex, amygdala, putamen, insula, and anterior cingulate. [^{11}C]CFN binding potential in such areas was negatively correlated with BMI and self-reported food addiction. However, there were no significant differences between healthy and obese subjects concerning the regional binding potentials of [^{11}C]raclopride (Karlsson et al. 2015). These results can be interpreted as evidence for downregulation of μOR in the human brain as a consequence of excessive stimulation by endogenous opioids, related to persistent overeating. However, a low density of μOR could also be a neurochemical trait that predisposes subjects to becoming obese.

In a follow-up study from the same year, group sizes were expanded to 25 obese and 20 healthy female subjects, and the correlation between regional μOR and D_2R binding potentials was examined (Tuominen et al. 2015). In healthy subjects, μOR and D_2R availabilities in ventral striatum and dorsal caudate nucleus were positively associated, and D_2R availability in ventral striatum was also associated with μOR availability in the ventral tegmental area. In obese subjects, an association between μOR and D_2R binding was observed in the caudate nucleus, but the association in the ventral striatum was much weaker than in lean subjects and the association between striatal D_2R and tegmental μOR binding was abolished. Thus, the interaction between the opioid and dopaminergic systems may be altered in obesity.

A second follow-up study addressed the question whether reduced μOR availability is a state or a trait in obesity. Sixteen obese women were scanned twice with [^{11}C]CFN and [^{11}C]raclopride, both before and 6 months after bariatric surgery (gastric bypass or sleeve gastrectomy). Measured binding potentials were compared to those of an age-matched healthy control group ($n = 14$). A reduced availability of

μ OR in ventral striatum, insula, amygdala, and thalamus was initially observed, but the binding potential of [11 C]CFN in these areas normalized after gastric surgery and weight loss (26.1 ± 7.6 kg). Apparently, the reduced availability of μ OR in the patient group was associated with the obese phenotype and was not a persistent neurochemical trait. In contrast to μ OR, the binding potential of D₂R was not altered after surgery (Karlsson et al. 2016).

21.5.7 Epilepsy

Opioid receptors and endogenous opioid peptides are known to play a role in the mechanisms underlying seizures. Many PET studies with opioid receptor ligands have been performed in patients with epilepsy. The first of these involved [11 C]CFN and subjects with complex partial seizures due to unilateral temporal seizure foci. [11 C]CFN binding in the temporal neocortex was greater on the side of the focus than on the contralateral side. This increase was interpreted as evidence for involvement of μ OR in a tonic anticonvulsant system that limits the spread of electrical activity (Frost et al. 1988). In contrast to [11 C]CFN binding, [11 C]DPN uptake (Mayberg et al. 1991) and [18 F]cyclofoxy (Theodore et al. 1992) uptake in the ipsilateral and contralateral lobes were not significantly different, suggesting that opioid receptor subtypes are differentially regulated in temporal lobe epilepsy. Evidence for differential regulation was obtained in a later study in which subjects with temporal lobe epilepsy were scanned both with the δ OR ligand [11 C]methylnaltrindole ([11 C]MeNTI) and with the μ OR ligand [11 C]CFN. Binding of both ligands was increased in the ipsilateral temporal cortex, but the regional pattern of the changes was different (more extended for [11 C]MeNTI than for [11 C]CFN). Upregulation of δ OR may indicate an anticonvulsant action for this receptor subtype (Madar et al. 1997). A more recent study examined [11 C]DPN binding in the brain of patients with temporal lobe epilepsy both shortly (within a few hours) after spontaneous epileptic seizures and interictally. In 14 healthy controls, no changes of [11 C]DPN binding were observed as a function of time, but in the patients, an increase of tracer binding was noted in the ipsilateral temporal pole and fusiform gyrus, shortly after seizures. The magnitude of this increase was inversely correlated to the interval that had elapsed since the last seizure, suggesting an increase of receptor binding during seizures followed by a gradual return to baseline (Hammers et al. 2007b). A follow-up article that was published after 6 years pointed out that in order to correctly quantify opioid receptor availability after spontaneous epileptic seizures, [11 C]DPN data need to be corrected for the partial volume effect. After such correction, post-ictal increases in tracer distribution volume could also be detected in the hippocampus (McGinnity et al. 2013). Thus, results from PET studies with three different ligands ([11 C]CFN, [11 C]MeNTI, and [11 C]DPN) have supported the idea that the opioid system is involved in seizure control.

The interictal distribution volume of [11 C]DPN in the cortex and thalamus of eight patients with childhood and juvenile absence epilepsy was found to be not significantly different from values in healthy age-matched controls; thus, there

appeared to be no overall abnormality of opioid receptors in this patient group (Prevett et al. 1994). To examine whether absence seizures are associated with release of endogenous opioids, investigators scanned eight patients with primary generalized epilepsy and eight control subjects with [^{11}C]DPN and PET. Serial absences were precipitated in the patients by hyperventilation for 10 min, starting 30–40 min after injection of the tracer. Increased washout of DPN was observed in the association cortex but not in other brain areas during seizures, as compared with control subjects and patients scanned without provocation of absences. Pharmacokinetic modeling suggested that absence seizures resulted in a significant, 15–41% decrease in the rate constant of association (k_3) of the tracer. These data were interpreted as evidence for the release of endogenous opioids in the association cortex at the time of absences, leading to increased opioid receptor occupancy (Bartenstein et al. 1993).

A later study involved five patients with reading epilepsy who were scanned both at baseline (reading a string of symbols) and during seizure activation (reading a scientific paper). The latter condition was associated with significantly lower binding of [^{11}C]DPN in the left parietotemporal-occipital cortex of the patients as compared with six healthy controls, suggesting release of endogenous opioids as part of a mechanism to terminate reading-induced seizures (Koepp et al. 1998).

Two patients with mesobasal temporal lobe epilepsy have been scanned with [^{11}C]DPN, before and after selective amygdalohippocampectomy. After removal of the epileptic focus, [^{11}C]DPN binding in the ipsilateral frontal cortex was found to be reduced, suggesting either downregulation of opioid receptors in the absence of seizures or postoperative neuronal dysfunction (Bartenstein et al. 1994).

21.5.8 Neurodegenerative Diseases

Losses of opioid receptors with different regional patterns have been observed in several neurodegenerative diseases.

21.5.8.1 Huntington's Disease

Significant decreases of [^{11}C]DPN binding (24–40%) were noted in the caudate and putamen of Huntington's disease (HD) patients using striatum-to-occipital cortex uptake ratios, spectral analysis, voxelwise parametric analysis, and statistical parametric mapping (SPM) for quantification (Weeks et al. 1997).

21.5.8.2 Alzheimer Disease

Decreases of opioid receptor binding (up to 40%) have been detected in the brain of Alzheimer disease (AD) patients using the tracer [^{18}F]cyclofoxy, and these changes were not correlated with decreases in regional cerebral blood flow (Cohen et al. 1997).

21.5.8.3 Parkinson Disease and Related Disorders

An initial study on eight clinically defined Parkinson disease (PD) patients, seven subjects with the striatonigral degeneration type of multiple system atrophy and six subjects with Steele-Richardson-Olszewski (SRO) syndrome examined opioid receptor binding in the human striatum with the tracer [^{11}C]DPN and PET. In the PD patient group, tracer binding was not significantly altered compared to healthy controls. Striatonigral degeneration was associated with reduced binding of [^{11}C]DPN in the putamen, but not in the caudate. In the SRO syndrome group, both caudate and putamen opioid receptor binding were significantly reduced. The binding pattern of [^{11}C]DPN may thus help to differentiate between various akinetic-rigid syndromes (Burn et al. 1995). In ten patients with the olivopontocerebellar variant of multiple system atrophy, a significant 12% reduction of the caudate-occipital ratio and a 15% reduction of the putamen-occipital uptake ratio of [^{11}C]DPN were noted. The latter decline was correlated to loss of [^{18}F]DOPA uptake in the putamen (average reduction 29%) (Rinne et al. 1995).

Using [^{11}C]DPN-PET and either a region-of-interest (ROI) or an SPM approach, a significantly reduced opioid receptor binding was later noted in the striatum and thalamus of PD patients with levodopa-induced dyskinesias but not in non-dyskinetic subjects (Piccini et al. 1997). No difference in striatal dopamine D_1 or D_2 receptor binding was found between the two subgroups, and measurements with PET and [^{15}O]water showed that rCBF after oral administration of levodopa was increased during dyskinesias in lentiform nuclei and motor, premotor, and dorsal prefrontal cortex. Thus, dyskinesias appear to arise not from a disturbance of dopamine receptor availability but rather from overactivity of opioid transmission, particularly the basal ganglia-frontal projections (Brooks et al. 2000).

In bilaterally 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned monkeys which had clinically recovered from the acute motor effects of dopaminergic neuron lesioning, a 65% decrease in the accumulation of [^{18}F]DOPA was noted in the basal ganglia. This decrease was associated with a 30–35% decline of [^{18}F]cyclofoxy binding in the caudate, anterior putamen, thalamus, and amygdala. The authors concluded that altered opioid receptor signaling (probably increased levels of Met-enkephalin) had contributed to the behavioral changes which were observed in the animals, i.e., the masking of their motor symptoms (Cohen et al. 1998). A later study from the same group showed that in unilaterally MPTP-lesioned monkeys with parkinsonian symptoms, opioid receptor availability is reduced by 30–35% on both the lesioned and the non-lesioned sides of the brain (Cohen et al. 1999).

In contrast to the decreases of opioid receptor binding which were observed in akinetic-rigid syndromes and PD patients with dyskinesias, no abnormalities of [^{11}C]DPN binding were noted in patients with primary torsion dystonia (carriers of the *DYT1* gene), and no correlation between the severity of dystonia and opioid binding could be detected (Whone et al. 2004).

21.5.9 Opioid Receptor Expression in Lung Tumors

A pilot PET study involving seven patients with lung carcinomas examined the feasibility of tumor imaging with the radioligands [^{11}C]MeNTI and [^{11}C]CFN. Four of these patients were also scanned with [^{18}F]FDG for clinical indications. All tumors showed an accumulation of opioid receptor ligands above background. The tumor-to-nontumor binding ratio of the δOR ligand [^{11}C]MeNTI (4.3 ± 1.3) was greater than that of the μOR ligand [^{11}C]CFN (2.4 ± 1.2) but lower than that of [^{18}F]FDG (7.7 ± 0.5). About 50% of [^{11}C]MeNTI uptake and 44% of [^{11}C]CFN uptake in the tumors could be blocked by naloxone. Particularly [^{11}C]MeNTI appears suitable for investigation of lung carcinoma biology, since δOR may be involved in tumor invasion and metastasis (Madar et al. 2007). A ligand targeting μOR and δOR was tested in tumor-bearing mice as a strategy for delivering cytotoxic drugs to lung cancer, but unfortunately, this strategy failed since the specificity of payload delivery was inadequate (Li and Low 2017).

21.6 Conclusion

PET imaging has provided insight in the involvement of opioid system in affective and sensory processing. Changes of opioid receptor expression related to increasing age and altered hormonal status have also been reported. Moreover, OR imaging has been successfully applied in the study of the pharmacokinetics and pharmacodynamics of novel and existing drugs.

Regional changes of OR availability have been observed in psychiatric and neurodegenerative disorders, epilepsy, pain, bulimia nervosa, behavioral addiction, and substance abuse. PET imaging may contribute to the development of improved techniques for the treatment of pain (and addiction) by monitoring the relationship between OR occupancy and symptom relief.

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