PET Radioligands for *In Vivo* Visualization of Neuroinflammation

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Abstract: Neuroinflammation is a well-orchestrated, dynamic, multicellular process playing a major role in neurodegenerative disorders. The microglia which make up the innate immune system of the central nervous system are key cellular mediators of neuroinflammatory processes. In normal condition they exert a protective function, providing tissue repair by releasing anti-inflammatory cytokines and neurotrophic factors. Upon neuronal injury or infection, they become overactivated, thereby releasing neurotoxic substances, amplifying neuroinflammation leading to neurodegeneration. Positron emission tomography (PET) provides a sensitive non-invasive imaging technique to study and quantify receptor and enzyme expression. A radiolabeled tracer for a protein (over)expressed in neuroinflammation and more specifically for the overactivated microglia would be useful as a diagnostic tool in the follow-up of neuroinflammation progression and to study the efficacy of anti-inflammatory therapy over time. In this manuscript, an overview of potential PET tracer targets upregulated during neuroinflammation is provided together with the current radiotracers used to image these targets. In addition, lead structures to develop radiotracers for new targets are suggested.

Keywords: Neuroinflammation, positron emission tomography.

1. INTRODUCTION

A. Neuroinflammation

Neuroinflammation is an adaptive response for restoring tissue homeostasis and implies a tight interplay between a tissue and the immune system. Examples of classical inflammation include the response to bacterial, parasitic or viral infections. It is a multicellular process characterized by (1) changes in local vasculature (increased blood flow and vascular permeability), (2) activation of resident immune competent cells, (3) infiltration of mobile cells of the immune system (neutrophils, macrophages and lymphocytes) and (4) cytokine production [1,2]. Neuroinflammation is the inflammation as observed in central nervous system (CNS) diseases including stroke, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), Parkinson’s disease (PD), Alzheimer’s disease (AD), Huntington’s disease (HD), viral/bacterial infections, neoplasias and head traumas. These pathologies trigger an immune activation in the brain which on the one hand is involved in tissue repair and neuroregeneration, and on the other hand results in collateral damage to brain tissue, loss of neurons and dysfunction. Neuroinflammation is associated with autoimmune diseases (e.g. MS), acute (e.g. ischemia) or chronic (e.g. AD) CNS disease processes and CNS infections (e.g. herpes simplex virus type 1 encephalitis). Neuroinflammation arising in the absence of microorganisms has been termed sterile inflammation [2-4]. Graeber et al. even proposed to replace the term ‘neuroinflammation’ with the term ‘microglial activation’ since the vast majority of neuroinflammatory conditions do not fulfill the main criteria of inflammation [1].

Microglia are the major resident immune cells of the CNS and constitute up to 10% of the total cell population of the brain. They exert both neuroprotective and neurotoxic roles. As a consequence of CNS insults e.g. deposition of amyloid beta (Aβ), plaques or stroke, microglia become overactivated and change from a resting, ramified form to a reactive, amoeboid form. The reactive, amoeboid form enables microglia to function as phagocytes, expresses new surface markers, proliferates and releases a variety of cytokines and growth factors. Activation of microglial cells is mediated by pattern recognition receptors (PRRs) that bind to pathogen associated molecular patterns (PAMPs) or damage associated molecular patterns (DAMPs) which are expressed on cells that play main roles in innate immune responses, including macrophages and glial cells. Toll-like receptors (TLRs) are an example of PRRs. They recognise various PAMPs which are not present in the host. For instance, TLR4 recognises lipopolysaccharide (LPS) originating from gram-negative bacteria, whereas TLR3 recognises viral double-stranded RNA [4]. Receptors for advanced glycation end-products (RAGE) which are correlated with Aβ interaction in AD [5,6] and NOD-like receptors (NLRs) which act as pivotal sensors of infection and stress in intracellular compartments [7], are other examples of PRRs. Microglia and astrocytes also express purinergic receptors which respond to ATP released from cells during cell death, traumatic injury, or ischemia [4,8]. In addition, scavenger receptors which are involved in the uptake of several substances such as oxidized proteins, lipids, apoptotic cells and which probably contribute to cell signaling are also expressed on microglia and astrocytes [4,9]. These receptors are supposed to be capable of detecting PAMPs and DAMPs such as Aβ and LPS. Recognition by and ligation of PRRs results in activation of signal transduction pathways which regulate diverse transcriptional and posttranscriptional processes. These include activation of downstream kinases such as MAP kinase which in turn control multiple, signal dependent transcription factors including NF-κB. These transcription factors regulate expression of an important subset of highly induced genes including cytokines. Genes encoding proteins with antimicrobial activities and genes influencing protein synthesis, substrate metabolism, cell motility, phagocytosis, intracellular killing and antigen presentation are also induced.

This wide array of biological changes which occur in activated microglia results in a communication network between the cells of the CNS immune response. Depending on the way of activation, microglia can be classified in two main groups: the M1 phenotype or classically activated microglia and the M2 phenotype or alternatively activated microglia. The pro-inflammatory phenotype M1 is activated by IFN-γ produced by Th1 T-lymphocytes or signaling through TLRs. The anti-inflammatory phenotype M2 is activated by IL-4 and IL-13 produced by Th2 T-lymphocytes and other cell types. M1-type microglia produce TNF-α, IL-1β, IL-6, nitric oxide, superoxide, hydrogen peroxide and matrix metalloproteinases with the purpose to defend the host against pathogens and tumor cells but unfortunately, these mediators cause also damage to healthy host cells. Besides these direct effects, the release of pro-
inflammatory cytokines by M1-type microglia also leads to an amplification of the inflammatory response and even a more injurious accumulation of neurotoxins in CNS tissue. In contrast, M2-type microglia produce IL-10 and arginase-1 leading to tissue remodeling and repair [2,4,10]. The phenotypic response of microglia to anti-inflammatory cytokines including IL-10 is described with the term “deactivated”. Microglia exhibit this deactivated phenotype in healthy conditions and may play a central role in maintenance of tissue homeostasis through communication with astrocytes and neurons. Several feedback mechanisms are described to attenuate inflammatory processes including induction of proteins that inhibit signal transduction pathways or anti-inflammatory cytokines (e.g. IL-10). All these actions allow microglia to serve as an immune surveillant in the CNS and they appear to be the main sensor of foreign signals recognized by PRRs. In response to this danger recognition, microglia secrete inflammatory mediators including TNF-α and IL-1β, which start the inflammatory cascade. Critical roles in establishing and maintaining inflammatory responses in neurodegenerative diseases are thus ascribed to microglial cells [4,11].

Neuroinflammation is related with many neurodegenerative diseases including AD, PD, ALS and MS. Increasing evidence suggests an active role of neuroinflammation in pathophysiology and disease progression as most neurodegenerative disorders are characterized by neuroinflammatory processes [4,12]. Specific for AD, the current hypothesis includes that activated microglia are protective in early stages of AD by promoting Aβ clearance but they become increasingly dysfunctional at later disease stages and consequently contribute to disease progression [12-18]. As the incidence of neurodegenerative disorders increases and treatment is in most cases only effective in the early stage of disease, diagnosis needs to be as early as possible. Because these neuropathologies are accompanied with neuroinflammation, detection of neuroinflammation is an interesting target for follow-up of disease progression and treatment. This highlights the importance of molecular (functional) imaging techniques, like PET, as they have the advantage of allowing detection of diseases in a much earlier stage than do structural imaging techniques.

### B. Positron Emission Tomography Imaging

Molecular imaging can be defined as a multidisciplinary field that aims to integrate patient-specific and disease-specific molecular information with traditional anatomical imaging readouts. In the past, in vivo imaging methods have largely been based on imaging gross anatomy. As a consequence, disease or treatment effects were mostly detected as structural abnormalities or morphological changes (= structural imaging). PET and single photon emission computed tomography (SPECT) allow to visualize molecular interactions using specific radiolabeled probes (= functional imaging). The main advantage of functional imaging over structural imaging is its potential for early detection of disease. In most pathologies, functional discrepancies appear in an earlier stage of disease than do structural deviations. For this reason, especially PET is extremely useful for early disease diagnosis.

PET is an in vivo molecular imaging technology based on tracers that are labeled with positron-emitting radionuclides (table 1). The spatial and temporal distribution of the tracer can be visualized by a PET camera. Non-invasive molecular imaging using PET provides information about protein localization and density and organ function in general. Briefly, positron emitting radionuclides are unstable due to an excess of protons in their nucleus. As a result a proton is converted to a neutron and a positron which is ejected from the nucleus and travels a short distance (depending on its kinetic energy) and combines with an electron in the surrounding tissue to form a positronium. The distance the positron travels, is called the positron range and results in an inherent image blur. The positronium will annihilate, thereby converting the mass of the particle into electromagnetic energy namely two gamma ray photons of 511 keV which are emitted over an angle of 180°. This pair of gamma rays can be detected by a ring of detectors and detection of multiple coincident 511-keV gamma rays enables reconstruction of a 3D image representing the quantitative distribution of the radionuclide [19,20]. The most commonly used radionuclides in PET tracer development are carbon-11 and fluorine-18. These radionuclides are produced in a cyclotron, usually by proton bombardment of nitrogen-14 and oxygen-18, respectively. Carbon-11 decays to boron-11 by positron emission and has a half-life of 20.4 min. Fluorine-18 decays to oxygen-18 by positron emission and has a half-life of 109.8 min. The short half-life of carbon-11 enables multiple scans on the same day in the same subject with the same or a different tracer, however on-site production using a cyclotron combined with a fast and efficient chemical incorporation into precursors is required. The half-life of fluorine-18 on the other hand is long enough to allow for longer and more complicated radiosynthesis and for transport of fluorine-18 labeled tracers from the site of production to remote nuclear medicine departments not equipped with a cyclotron.

The ideal PET tracer should exhibit following characteristics: it should have a high affinity for the target, i.e. having an equilibrium dissociation constant (K_D) value in the subnanomolar to low nanomolar range. The affinity of the tracer for its target should be at least 5 to 10-fold higher than the target (receptor) expression (B_max). Also a high selectivity for the target is required, preferably 100-fold less affinity for any other binding site with the same expression level [21].

The blood-brain barrier (BBB) is a major hurdle in the development of drugs targeting the brain. Passive transfer across the BBB is promoted by low molecular mass (< 500 Da), a polar surface area (PSA) less than 80 Å², lack of a formal charge at physiological pH and a logD Mayor (distribution coefficient at pH 7.4) in the approximate range 1-3. Large and hydrophilic molecules are therefore excluded from penetration or diffusion into the brain, except if a specific carrier for such a molecule exists. In addition, tracer uptake in the brain is influenced by efflux transporters like P-glycoproteins which are highly prevalent at the BBB. Substrates for P-glycoproteins are in general highly lipophilic, carry a positive charge at pH 7.4 and have multiple aromatic groups [21,22]. Highly lipophilic molecules usually also show a high non-specific binding to lipophilic membranes and proteins in vivo. The slow protein dissociation of tracers with high in vivo plasma protein binding hampers the brain uptake of these tracers as only the unbound fraction can cross the BBB. Moreover, highly lipophilic tracers complicate formulation for intravenous injection due to low solubility in water and adsorption of molecules on the surface of vials and syringes. After crossing the BBB, the ideal tracer should show low non-specific binding in the brain, reversible (allowing an equilibrium binding and kinetic modeling) and specific target binding, no toxicity and no metabolism in the brain or formation of lipophilic radiometabolites that cross the BBB. As a PET camera cannot discriminate between different chemical forms of the positron emitting radionuclide, the presence of radiometabolites in the brain contaminates the specificity of the tracer signal in the CNS and hinders accurate quantification [21].

Nuclear imaging in general also has some limitations including patient exposure to ionizing radiation, poor anatomical information and a low resolution compared to other molecular imaging modalities. The latter constraints are resolved by coupling functional imaging to structural imaging (= hybrid or fusion-imaging). This approach introduced magnetic resonance imaging (MRI) and computed tomography (CT) fusion systems (PET-MRI/CT, SPECT-MRI/CT). In these fusion systems, the higher-resolution anatomical detail from MRI or CT compensates for the lower-resolution molecular information from PET or SPECT. The combination of the
two techniques will play an increasing role in clinical molecular imaging [24].

2. PET IMAGING OF NEUROINFLAMMATION

Neuroinflammation is expected to contribute to several human brain disorders, including cerebral ischemia, trauma, spinal cord injury, MS, acquired immune deficiency syndrome (AIDS) dementia, PD and AD. Although variations in glucose metabolism and cerebral blood flow have successfully been exploited to detect neuroinflammation using PET and SPECT, changes in these parameters lack specificity for neuroinflammation as they can be induced by other causes as well (e.g. hypercapnia increases cerebral blood flow). Several physiological parameters are altered during cerebral inflammation and these indicators are relatively specific for neuroinflammation and will be further discussed below, together with an overview of the reported PET tracers developed for these more specific neuroinflammatory targets.

A. Translocator Protein (TSPO)

The TSPO receptor, also called the peripheral benzodiazepine receptor (PBR), is one of the most studied biomarkers of neuroinflammation. The abbreviation TSPO has been taken over from the corresponding protein found in the outer mitochondrial membrane of bacteria, i.e. tryptophan-rich sensory protein (TspO). Two benzodiazepine receptors are expressed in humans. The central benzodiazepine receptor is coupled to the $\gamma$-aminobutyric acid A receptor (GABA$_A$R) and modulates GABA-regulated opening of Cl$^-$ channels and inhibition of neuronal activity. The PBR receptor or TSPO receptor is an 18-kDa protein consisting of 169 amino acids. TSPO is part of a multimeric complex existing of the 32-kDa voltage-dependent anion channel (VDAC) and the 30-kDa adenine nucleotide carrier (ANC) located in the outer mitochondrial membrane of microglial cells [25,26]. A variety of physiological functions such as cell growth and proliferation, bile acid synthesis, calcium flow, chemotaxis and cellular immunity, heme biosynthesis, mitochondrial respiration, apoptosis and steroidogenesis are ascribed to TSPO. In addition, TSPO plays a crucial role in neurosteroidogenesis and more specifically in the transmembrane transport of cholesterol from the outer to the inner mitochondrial membrane, where the side chain cleavage by cytochrome p450 converts cholesterol into pregnenolone. Pregnenolone is an important precursor for cerebral steroids which play a crucial role in brain development and normal functioning during adulthood. Because of the correlation between TSPO activation and stimulation of neurosteroid synthesis, TSPO ligands are potential therapeutic agents for brain injury and inflammation. Under healthy circumstances, TSPO levels in the CNS are low. In response to brain inflammation, TSPO levels increase dramatically in glial cells as detected in several neurodegenerative diseases such as PD, ALS, HD and AD. Literature suggests that cytokines might play a role in increasing TSPO expression in astrocytes. In contrast to microglia (early up-regulation after injury), astrocytes display a delayed, but more persistent induction of TSPO expression during astrogliosis which appears at later time points after the onset of brain injury [12,25,27,28].

Since the response of TSPO to injury is correlated with the degree of damage in neuroinflammation, TSPO has been identified as a promising biomarker for neuroinflammation and the number of PET radioligand candidates for TSPO has significantly increased in the last years (Fig. 1). The most studied TSPO-radioligand is \[^{11}C\]PK11195, of which the R-enantiomer has an approximately 2-fold higher affinity for TSPO compared to the S-enantiomer. This isoquinoline derivative with nanomolar affinity for TSPO shows an increased binding in affected brain areas in neuroinflammatory conditions.

### Table 1. Radionuclides used in PET [23]

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Half-life production</th>
<th>Mode of decay (%)</th>
<th>E$_{\gamma}$ max (keV) max. range in H$_2$O (mm)</th>
<th>chemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{11}$C</td>
<td>20.4 min Cyclotron</td>
<td>$\beta^+$ (100%)</td>
<td>960 keV 3.9 mm</td>
<td>Fast organic chemistry</td>
</tr>
<tr>
<td>$^{13}$N</td>
<td>9.97 min Cyclotron</td>
<td>$\beta^+$ (100%)</td>
<td>1198 keV 5.1 mm</td>
<td>Fast organic chemistry</td>
</tr>
<tr>
<td>$^{15}$O</td>
<td>2.03 min Cyclotron</td>
<td>$\beta^+$ (100%)</td>
<td>1732 keV 8.0 mm</td>
<td>Fast on-line gas phase chemistry</td>
</tr>
<tr>
<td>$^{18}$F</td>
<td>109.8 min Cyclotron</td>
<td>$\beta^+$ (97%)</td>
<td>634 keV 2.3 mm</td>
<td>Fast organic chemistry</td>
</tr>
<tr>
<td>$^{64}$Cu</td>
<td>12.7 h Cyclotron</td>
<td>$\beta^+$ (100%)</td>
<td>653 keV 2.4 mm</td>
<td>Chelation chemistry</td>
</tr>
<tr>
<td>$^{68}$Ga</td>
<td>68 min Generator</td>
<td>$\beta^+$ (89%)</td>
<td>1899 keV 8.9 mm</td>
<td>Chelation chemistry</td>
</tr>
<tr>
<td>$^{89}$Zr</td>
<td>78.4 h Cyclotron</td>
<td>$\beta^+$ (100%)</td>
<td>897 keV 3.6 mm</td>
<td>Chelation chemistry</td>
</tr>
<tr>
<td>$^{124}$I</td>
<td>4.17 d cyclotron</td>
<td>$\beta^+$ (23%)</td>
<td>1535 keV (50 %) 6.9 mm</td>
<td>Organic chemistry</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\beta^+$ (77%)</td>
<td>2138 keV (50 %) 10.2 mm</td>
<td></td>
</tr>
</tbody>
</table>
animal models as well as in humans. Although both microglia and astrocytes overexpress TSPO, a majority of the evidence suggests that \(^{11}\text{C}\)PK11195 binding in neurological diseases is increased in microglia with lower or less significant contributions from astrocytes. Until a few years ago it was the only PET tracer applied for imaging microglia activation in humans. Although \(^{11}\text{C}\)PK11195 has several limitations such as a high level of nonspecific binding and poor signal-to-noise ratios complicating its quantification, it is still used as a TSPO imaging agent by several research groups. For example, zymosan-induced (foam white matter lesion) microglial activation and its response to minocycline was quantitatively imaged in rat brain using \(^{11}\text{C}\)PK11195 [29]. Folkersma et al. observed an increased cerebrovascular uptake of this tracer ten days after traumatic brain injury in rats [30]. The widespread and prolonged increase in \(^{11}\text{C}\)PK11195 binding was also observed in patients until six months after traumatic brain injury [31]. Kumar et al. demonstrated an increased \(^{11}\text{C}\)PK11195 uptake with age in humans [32]. Increased \(^{11}\text{C}\)PK11195 binding was also observed in the cortex of patients with MS. The binding correlated with the degree of disability as assessed by the expanded disability status scale (EDSS) and the multiple sclerosis impact scale (MSIS) [33,34]. Other recent studies using \(^{11}\text{C}\)PK11195 have been performed by Gulyás et al. [35], Hughes et al. [36], Ren et al. [37], Rapic et al. [38], Garvey et al. [39], Boutin et al. [40] and Dickens et al. [41]. For an overview of in vitro PET imaging studies using \(^{11}\text{C}\)PK11195, the reader is referred to reviews of Venneti et al. [28], Chauveau et al. [42] and Owen and Matthews [43]. An overview of studies with other TSPO tracers is given in table 2. Note that for \(^{11}\text{C}\)CLINME and \(^{18}\text{F}\)FGE-180, Ki values are given in table 2 as there are no data available in literature of binding affinity studies where these tracers are compared to PK11195 in the same assay.

 Recently, Owen et al. [85,86] reported a TSPO polymorphism in man with a trimodal distribution in binding affinity (high-affinity binders, low-affinity binders and mixed affinity binders) for several TSPO ligands. Differences in affinity between high-affinity binders and low-affinity binders were approximately 50-fold with PBR28, approximately 17-fold with PBR06 and approximately 4-fold with DAA1106, DPA713 and PBR111. The consequence of this polymorphism is that knowledge of binding status is needed to correctly quantify TSPO expression using these PET ligands [85,86]. The reader should keep this in mind when looking at the affinities compared to PK11195 shown in table 2.

For a more detailed discussion on TSPO (PET) ligands, the reader is referred to some other reviews [42,43,87,88].

**B. Cannabinoid 2 Receptor (CB₂R)**

A second important target in neuroinflammation is the CB₂R. Two cannabinoid receptors have been described in literature: CB₁R and CB₂R. These receptors are \(G_\text{q/11}\) protein coupled receptors, which upon activation inhibit adenylyl cyclase and stimulate mitogen-activated protein kinases (MAPKs). Activation of CB₁R also leads to an inhibition of voltage-gated calcium channels and stimulation of inwardly rectifying potassium channels, whereas activation of CB₂R is coupled to an increased release of ceramide. Both receptors are involved in a release of nitric oxide and a subsequent activation of cyclic guanosine monophosphate (cGMP) levels [89]. The CB₂R is related to organs and tissues of the immune system with high expression in tonsils and spleen, whereas the CB₁R is mainly expressed in the central nervous system [90,91]. The CB₂R is upregulated in certain pathological conditions such as cancer, atherosclerosis, peripheral/central inflammation and several brain disorders [92-102]. Prolonged oral cannabinoid administration has been proposed to prevent neuroinflammation, result in lower Aβ levels and improve cognitive performance in Tg APP 2576 mice [103]. Microglial cells show very low CB₂R expression in basal conditions whereas in CNS pathologies the CB₂R expression is significantly increased. In experimental autoimmune encephalomyelitis (EAE), the expression of CB₂R is 10 times higher in activated microglia compared to basal levels [95,97].

The CB₂R is an interesting target for visualization of neuroinflammation as its expression in brain is very low in control conditions, but is upregulated in activated microglia. Evans et al. [104] synthesized and evaluated \(^{11}\text{C}\)methoxy-Sch225336 (Fig. 2). Although this tracer displays low nanomolar affinity, its limited brain uptake makes this tracer unfavorable for imaging CB₂R expression in the brain [104]. Next, Evans et al. [105] synthesized carbon-11 and fluorine-18 labeled 2-oxoquinoline derivatives \(^{11}\text{C}\)NE40 and \(^{18}\text{F}\)oxoquinoline A (Fig. 2) with nanomolar affinity for the human CB₂R. Both tracers were first evaluated in mice. Biodistribution studies showed uptake and washout of both tracers from brain whereas persistent accumulation was observed in the spleen. *In vitro* and *ex vivo* autoradiography confirmed the specificity of the CB₂R binding in the spleen. Both tracers were rapidly metabolized in plasma in *vivo*, whereas only a relatively small amount of radiometabolites was detected in brain [105]. \(^{11}\text{C}\)NE40 was then further evaluated in normal Wistar rats. The CB₂R specific spleen retention of \(^{11}\text{C}\)NE40 observed in the mice was even more pronounced in the rat biodistribution studies. *Ex vivo* autoradiography confirmed that the binding to rat spleen was specific for CB₂R. MicroPET studies in normal mouse, rat and rhesus monkey displayed relatively high brain uptake and moderately fast brain washout. No toxic (toxicity study in Wistar rats) or mutagenic (Ames test) effects were observed for NE40. Reversible binding to human CB₂R (hCB₂R) was confirmed by a microPET chase study with GW405833 (a nonstructurally related CB₂R partial agonist) in a rat model with local overexpression of hCB₂R [106,107]. Turkman et al. [108] synthesized and tested a library of new fluorinated 2-oxoquinoline CB₂R ligands with affinities in the nanomolar range [108]. *In vitro* autoradiography on tissues of CB₂R expressing tumor bearing mice and *in vitro* binding assays with CB₂R expressing cell lysate showed that one of the fluorine-18 labeled oxoquinoline compounds, \(^{18}\text{F}\)oxoquinoline B (Fig. 2), bound in a specific way to CB₂R. Unfortunately, its poor solubility hampered *in vivo* studies [109]. \(^{11}\text{C}\)A-836339 (Fig. 2), developed by Horti et al. [110], shows subnanomolar affinity for CB₂R and exhibits high specific CB₂R cerebral uptake in a LPS-induced mouse model of neuroinflammation and in a mouse model of AD [110]. GW405833, a known CB₂R partial agonist, was radiolabeled with \(^{18}\text{F}\)fluoroethyl bromide to introduce the fluorine-18 label via a fluoroethoxy chain. *In vitro* studies showed that \(^{18}\text{F}\)FJE-GW405833 (Fig. 2) has nanomolar affinity and selectivity for CB₂R. However, the presence of a large fraction of radiometabolites in brain arising from the loss and metabolism of the \(^{18}\text{F}\)fluoroethoxy chain, limits its use for imaging neuroinflammation [111]. The carbon-11 labeled derivative \(^{11}\text{C}\)GW405833 (Fig. 2) on the other hand showed high BBB penetration, lower fraction of radiometabolites in brain, and reversible and specific CB₂R *in vivo* binding in an animal model with local overexpression of CB₂R in the brain [106]. Other radiolabeled high affinity CB₂R ligands with no or limited *in vivo* evaluation will be discussed briefly. Triaryl ligands for the CB₂R with (sub)nanomolar affinity, synthesized by Fujinaga et al. [112], were labeled with carbon-11 and showed relatively high uptake in mouse brain [112]. Gao et al. [113] used quinoline derivatives as candidate PET radioligands for CB₂R imaging. Three compounds with nanomolar affinity and high selectivity for CB₂R were efficiently labeled with carbon-11 but unfortunately no *in vivo* studies were performed [113]. Rühl et al. [114] synthesized a number of N-aryl-oxa-diazoyl-propionamides with nanomolar affinity and selectivity for CB₂R over CB₁R. Imaging studies with the fluorine-18 labeled analogs are in progress [114]. Teodoro et al. [115] radiolabeled N-aryl-oxa-diazoyl-propionamides successfully with fluorine-18 but the main radiometabolites were found to cross the BBB in mice.
Table 2. Overview of studies with TSPO tracers

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Affinity (compared to PK11195)</th>
<th>Animal model/human pathology/experiments</th>
<th>Increased TSPO expression (yes or no)? Other information (in italics)?</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{[1]}\text{C})DAA1106</td>
<td>5-6 fold higher</td>
<td>Stereotactic LPS, 6-OHDA lesioned rat models</td>
<td>Yes</td>
<td>[44]</td>
</tr>
<tr>
<td>(^{[1]}\text{C})PBR28</td>
<td>5-6 fold higher</td>
<td>Cerebral infarction rat</td>
<td>Yes</td>
<td>[45]</td>
</tr>
<tr>
<td>(^{[1]}\text{C})PBR28</td>
<td>5-6 fold higher</td>
<td>Systemic LPS non-human primates</td>
<td>Yes</td>
<td>[46]</td>
</tr>
<tr>
<td>(^{[1]}\text{C})PBR28</td>
<td>5-6 fold higher</td>
<td>Non-human primate, comparison with (^{[1]}\text{C})PK11195</td>
<td>Yes Higher TSPO specific signal, less metabolism</td>
<td>[47]</td>
</tr>
<tr>
<td>(^{[1]}\text{C})PBR28</td>
<td>5-6 fold higher</td>
<td>Patients with temporal lobe epilepsy</td>
<td>Yes</td>
<td>[48]</td>
</tr>
<tr>
<td>(^{[1]}\text{C})PBR28</td>
<td>5-6 fold higher</td>
<td>Radiation dosimetry, kinetic analysis in non-human primates and man</td>
<td>/</td>
<td>[49],[50],[51]</td>
</tr>
<tr>
<td>(^{[1]}\text{C})PBR28</td>
<td>5-6 fold higher</td>
<td>Patients with Alzheimer’s disease</td>
<td>Yes</td>
<td>[52]</td>
</tr>
<tr>
<td>(^{[1]}\text{C})PBR28</td>
<td>5-6 fold higher</td>
<td>Patients with mild-to-moderate depression</td>
<td>Yes</td>
<td>[53]</td>
</tr>
<tr>
<td>(^{[18]}\text{F})FEPPA</td>
<td>18 fold higher</td>
<td>Metabolite study in rat</td>
<td>Few metabolites in brain</td>
<td>[54]</td>
</tr>
<tr>
<td>(^{[18]}\text{F})PBR28</td>
<td>2 fold higher</td>
<td>Quantitation in human brain; whole body distribution and radiation dosimetry in human</td>
<td>/</td>
<td>[55],[56]</td>
</tr>
<tr>
<td>(^{[18]}\text{F})PBR28</td>
<td>2 fold higher</td>
<td>Healthy elderly individuals</td>
<td>No</td>
<td>[57]</td>
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<tr>
<td>(^{[18]}\text{F})PBR28</td>
<td>2 fold higher</td>
<td>Stereotactic AMPA rat model</td>
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<td>[58]</td>
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<tr>
<td>(^{[18]}\text{F})FMDAA1106</td>
<td>5-6 fold higher</td>
<td>WT rats</td>
<td>Specific binding</td>
<td>[60]</td>
</tr>
<tr>
<td>(^{[18]}\text{F})FMDAA1106</td>
<td>5-6 fold higher</td>
<td>Non-human primate, metabolite study in mice</td>
<td>/</td>
<td>[61]</td>
</tr>
<tr>
<td>(^{[18]}\text{F})FEDAA1106</td>
<td>8 fold higher</td>
<td>WT rat</td>
<td>Specific binding</td>
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<td>8 fold higher</td>
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<td>/</td>
<td>[61]</td>
</tr>
<tr>
<td>(^{[18]}\text{F})FEDAA1106</td>
<td>8 fold higher</td>
<td>MS patients</td>
<td>High uptake in occipital corte, no metabolites in mouse brain</td>
<td>[62]</td>
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COX is a intracellular key enzyme in the conversion of arachidonic acid to prostaglandins (PGs). Both lipid compounds are involved in several physiological and pathological processes, including (neuro)inflammation [118] indicating a key role of COX in the inflammatory cascade. Another indication for the role of COX in neuroinflammation is the fact that the use of nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit COX activity, is correlated with a reduced risk of developing AD later in life [119,120].

Although viewpoint varies [126-128], COX-2 is described in several physiological and pathological conditions [138-142]. Overexpression of COX-1 and COX-2 has been reported in patients with ischemia, traumatic brain injury and in several neurodegenerative diseases such as AD, PD and ALS. These findings suggest that COX is a critical component in the neuroinflammatory processes associated with neurodegeneration [143-146].

In search of an appropriate PET tracer for COX-2 imaging some COX-2 inhibitors have been used as scaffold. One of the first developed PET tracers to image COX-2 was [18F]arachidonic acid. However, PET studies showed incorporation of the tracer into phospholipids in the brain as expected since it is a building block for membrane lipids, making it unsuitable for COX-2 imaging [147-149]. Although favourable in vitro results were obtained with [18F]SC58125, this tracer lacked specific binding in vivo [149,150]. Also for [18F]desbromo-DuP-697, PET studies in pig showed absence of specific binding in the brain [149,151]. The [18F]fluoromethyl analogue of valdecoxib, synthesized by Toyokuni et al. [152] was characterized by rapid defluorination in vivo in mice [152]. In vivo defluorination was also observed with [18F]celecoxib derivative A (Fig. 3) in rats. In baboons, however, defluorination was less pronounced and the distribution of the radioligand in baboon brain was consistent with the known distribution of COX-2. Nevertheless, because of the poor specific activity of [18F]celecoxib an improved radiolabeling method should be developed [153].

Rofecoxib was labeled with carbon-11 by de Vries et al. (Fig. 3) [154]. Although there was correlation between [11C]rofecoxib uptake and COX-2 distribution in healthy rats, [11C]rofecoxib could not detect COX-2 expression in two different rat models of inflammation [154]. Comley et al. [155] studied uptake and regional distribution of [15C]celecoxib in human brain and observed a heterogeneous distribution of the radiotracer [155]. Uddin et al. [156] evaluated a number of fluorinated derivatives of indomethacin and celecoxib as COX-2 PET imaging agents. A fluorinated derivative of celecoxib, [15C]celecoxib derivative B (Fig. 3), appeared to be the most promising lead. The radioligand showed sufficient metabolic and radiochemical stability to distribute to xenograft tumors and inflammatory lesions and exhibited highly selective uptake in inflammatory tissue and tumors compared to surrounding normal tissue in mice and rats [156].

In addition, studies have also indicated a previously unrecognized proinflammatory role of COX-1 in the pathophysiology of acute and chronic neurological disorders [138-142]. Overexpression of COX-1 and COX-2 has been reported in patients with ischemia, traumatic brain injury and in several neurodegenerative diseases such as AD, PD and ALS. These findings suggest that COX is a critical component in the neuroinflammatory processes associated with neurodegeneration [143-146].

**Table 2** Contd….

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</tbody>
</table>

*personal communication H. Boutin*

[115]. Radiolabeling and *in vitro*/*in vivo* evaluation of N-(1-adamantyl)-8-methoxy-4-oxo-1-phenyl-1,4-dihydroquinoline-3-carboxamide is performed by Mu *et al.* [116]. This radiotracer showed promising *in vitro* characteristics but further *in vivo* research is needed [116]. A triazine derivative was radiolabeled with fluorine-18 by Hortala *et al.* [117]. This radiotracer has nanomolar affinity for the human CB2R and was selective versus human CB1R [117]. The reader is referred to the review of Evens and Bormans [21] for a complete overview of non-invasive imaging of the CB2R using PET before 2010 [21].

**C. Cyclooxygenase (COX)**

COX is a intracellular key enzyme in the conversion of arachidonic acid to prostaglandins (PGs). Both lipid compounds are involved in several physiological and pathological processes, including (neuro)inflammation [118] indicating a key role of COX in the inflammatory cascade. Another indication for the role of COX in neuroinflammation is the fact that the use of nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit COX activity, is correlated with a reduced risk of developing AD later in life [119,120]. Several studies have confirmed these observations although some well-designed studies have found different results. Therefore Breetner et al. [121] analyzed the association of prior NSAID exposure with incident dementia and AD in an elderly community-based cohort. In contrast to the hypothesis that NSAIDs protect against dementia and AD, the authors observed an increased incidence of dementia and AD in heavy NSAID users [121]. Also in PD, neuroprotective characteristics have been ascribed to NSAIDs [122,123], although recent studies could not confirm this [124,125]. Two distinct COX isoforms, COX-1 and COX-2, have been characterized [126-128]. Along with COX-1 and COX-2, COX-3 is described in literature. COX-3 is a COX-1 splice variant inhibited by acetaminophen and other analgesic/antiinflammatory drugs [129]. COX-1 is constitutively expressed in most tissues and is considered responsible for homeostatic PG synthesis [130] whereas COX-2 is an enzyme inducible by inflammation. This led to the development of (selective) COX-2 inhibitors which do not disturb the physiological functions of COX-1-derived PGs. The expression of COX-2 is strongly elevated in brain by inflammatory stimuli and this overexpression is predominantly located in activated microglia and astrocytes [131]. Furthermore, increased COX-2 expression is implicated in a wide variety of cancers, including colorectal adenocarcinoma, breast and lung cancer [132-135]. COX-2 is also constitutively expressed in the CNS, where it has a pivotal role in synaptic activity, long-term synaptic plasticity [136,137] and in the neurovascular coupling during functional hyperemia [136]. In addition…
esters showed high BBB penetration. Radiometabolite analysis of \([^{11}C]\)ketoprofen methyl ester in rat brain showed conversion into the pharmacologically active form \([^{11}C]\)ketoprofen (Fig. 3). \([^{11}C]\)Naproxen methyl ester, \([^{11}C]\)flurbiprofen methyl ester, \([^{11}C]\)fenoprofen methyl ester, \([^{11}C]\)ketoprofen methyl ester and \([^{11}C]\)loxoprofen methyl ester all showed higher uptake into the lesioned hemisphere of an LPS-induced neuroinflammation rat model compared to the contralateral nontreated area. More than 90% of the proradiotracer was hydrolyzed after 5 min in rat brain. This first hydrolase activity step should be kept in mind when analyzing PET images. In order to determine the binding specificity of 2-aryl\([^{11}C]\)propionic acid methyl esters as proradiotracer in the inflamed area, the authors performed blocking microPET and \textit{ex vivo} autoradiography experiments with \([^{11}C]\)ketoprofen methyl ester. Co-injection of the cold ketoprofen methyl ester resulted in a significant reduction of radioactivity in the area of LPS-induced inflammation. The authors conclude that this indicates that \([^{11}C]\)ketoprofen binds specifically to COX-2 [157] but it is not clear whether they took into account the first step hydrolysis of the proradiotracer. Probably hydrolase activity is measured instead of COX-2 activity.

**D. Matrix Metalloproteinase (MMP)**

MMPs are secreted extracellular targets for imaging of neuroinflammation. As their name implies, MMPs are involved in turnover and degradation of the extracellular matrix. They belong to a family
of zinc- and calcium-dependent endopeptidases. Besides degradation of the extracellular matrix, MMPs act on pro-inflammatory cytokines, chemokines and other inflammation related proteins [158,159]. MMPs are synthesized as inactive zymogens and have a very low expression level in healthy tissues whereas elevated MMP expression has been found in disease processes like atherosclerosis, tumor cell metastasis and inflammation [158,160-162]. MMP activity is controled at four levels: gene expression, compartmentalization, zymogen activation and by endogenous tissue inhibitors of metalloproteinases (TIMPs) [158,163]. Vos et al. [164] showed expression of MMP-12 in phagocytotic macrophages in active MS lesions (human tissue) indicating a role for MMP-12 during demyelination in MS [164]. Nuttall et al. [165] activated human microglial cells with LPS resulting in a significant increase in mRNA levels of MMP-1, MMP-3, MMP-8, MMP-10 and MMP-12 [165]. Upregulation of MMP-12 expression was also observed in a mouse model of EAE with disease progression together with the expression of other inflammation related cytokines [166]. Crocker et al. [167] compared MMP expression profiles in cultures of astrocytes and microglia. Astrocytes extracted from mouse brain constitutively expressed MMP-11, MMP-14 and MMP-2 and showed induction of MMP-3 in response to IL-1β but did not respond to LPS. In contrast, microglia extracted from mouse brain constitutively expressed high levels of MMP-12 and showed strong induction of MMP-9 and MMP-14 in response to LPS [167]. Liu et al. [168] described MMP-12 as an inflammatory activating marker which is upregulated in mouse brain during normal aging. MMP-12 should facilitate recruitment of bone marrow-derived microglia into the brain which could be a mechanism to enhance aging-associated neuroinflammation [168]. These articles indicate that MMPs play an important role during (neuro)inflammation. Although overexpression of many MMPs in CNS pathology is correlated with detrimental effects, MMPs also have various roles in beneficial activities as in neurogenesis, axonal growth and myelinogenesis. Whether MMPs become beneficial or detrimental after nervous system injuries is determined by the stage of the CNS injury, the type of injury and the pathophysiology of the disorder. For more information on MMPs and their (beneficial/detrimental) role in CNS, the reader is referred to Yong et al. [169].

Two approaches are being used for imaging MMPs. One approach is based on use of radiolabeled peptides or proteins acting as matrix metalloproteinase inhibitors (MMPIs). Another approach is based on radiolabeled small molecular mass non-peptidyl MMPIs [163]. Wagner et al. [159] published an overview of the PET ligands developed for MMP visualization before 2006. Although the focus in this review is mainly on PET/SPECT imaging of MMPs during cancer and atherosclerosis, the lead structures shown could also be used as a template to develop radiotracers for imaging of neuroinflammation [159]. The N-sulfonyl amino acid hydroxamate CGS 25966 and CGS 27023A (Fig. 4) are broad-spectrum inhibitors of MMP-1, MMP-2, MMP-3 and MMP-9 in the nanomolar range and most radiolabeled MMPIs have been designed based on these lead structures [159]. Fluorine-18 labeled derivatives of CGS 27023A and CGS 25966, [18F]CGS 27023 A 1 and [18F]CGS 25966 1 (Fig. 4), were synthesized and the non-radioactive compounds displayed IC50 values in the low nanomolar range. Biodistribution studies of both tracers in WT mice did not show tissue specific accumulation. The authors suggest that this low uptake in normal mice could be an advantage in studying activated and dysregulated MMPs but validation of the tracers in animal models of

![Fig. (2). CB2R PET tracers.](image_url)
neuroinflammation has not been reported [163,170]. Another \(^{18}\)F-labeled derivative of CGS 27023A (\(^{18}\)F)CGS 27023 A 2, Fig. 4) was also developed by Wagner et al. [163]. This compound showed IC\(_{50}\)-values in the nanomolar and subnanomolar range for MMP-2, MMP-8, MMP-9 and MMP-13. Initial small-animal PET in vivo studies in WT mice did not show any unfavourable aspecific tracer accumulation nor accumulation of free \(^{18}\)F[fluoride in bone [163]. Breyleholtz et al. [171] designed barbiturate-based radiotracers for non-invasive imaging of MMPs. These pyrimidine-2,4,6-triones of which the structure is based on RO 28-2653 (Fig. 4) exhibit specific activity for subgroups of secreted MMPs and have higher metabolic stability compared to hydroxamic acid-based MMP inhibitors, such as CGS 27023A. Biodistribution studies in WT mice of a fluorine-18 labeled triazole-substituted hydroxamate (Fig. 4) in vitro characterization of a new series of fluorinated pyrimidine-2,4,6-trione-based MMPIs. One of these MMPI. \(^{18}\)F[RO 28-2653 derivative 2 (Fig. 4), was successfully radiolabeled with fluorine-18 with high radiochemical yield and showed high in vivo stability. The IC\(_{50}\)-values for MMP-2 and MMP-9 were in the higher nanomolar range and the logD was 0.78 ± 0.02 which makes it a suitable PET tracer to image peripheral MMP expression but unsuitable for MMP imaging in the brain. In a next step preclinical PET/CT studies will be performed in animal models with known MMP up-regulation [172]. Recently, a fluorine-18 labeled triazole-substituted hydroxamate (Fig. 4) with nanomolar IC\(_{50}\)-values (0.006 - 107 nM) for MMP-2, MMP-8, MMP-9 and MMP-13 was reported by Hugenberg et al. [173]. The tracer showed high stability in vitro in mouse and human plasma. In vivo one polar radiometabolite was detected at 30 min post tracer injection. Biodistribution was studied in vivo with a microPET experiment in WT mice showing fast plasma clearance and absence of aspecific binding and defluorination. The tracer will be further evaluated in murine disease models with upregulated levels of activated MMPs [173]. Most compounds displayed in (Fig. 4) are hydrophilic and this means difficult BBB penetration. Upon evaluation of these compounds in animal models of neuroinflammation, high brain uptake could be due to BBB damage.

E. Monoamine Oxidase-B (MAO-B)

Although this review mainly covers targets that are localized in microglia, astrocytes also express some proteins during neuroinflammation which could be imaged by PET such as MAO-B. MAOs catalyze oxidative deamination of neurotransmitters and thus regulate their availability and physiological activity. Consequently, they are an important target for therapeutic drugs. More specifically, MAO-B inhibitors such as deprenyl are used to treat PD as they increase dopamine concentration in brain by inhibiting breakdown of dopamine. MAO-B is of special interest in imaging neuroinflammation because the MAO-B enzyme is upregulated in reactive astrocytes during neuroinflammation [35]. Several PET tracers have been proposed with structures derived from known MAO-B-inhibitors.

Carbon-11 labeled L-deprenyl (Fig. 5) has been successfully used as a PET radioligand to image increased MAO-B activity in CNS diseases with neuroinflammation including pituitary adenoma [174], PD [175], focal epilepsy [176,177], traumatic brain injury [178], ALS [179] and AD [35,180]. Saba et al. [181] characterized \(^{18}\)F]Fluorodeprenyl (Fig. 5) was evaluated by Vassev et al. [182] in WT rats and showed relatively high brain uptake that reflected regional MAO-B density in the brain and displayed moderate dose-dependent blocking with L-deprenyl. However, the signal-to-noise ratio was too low for further in vivo imaging studies [182]. \(^{18}\)F]Fluorodopamine (\(R = \text{CH}_3\) \(^{11}\)C]ketoprofen methyl ester \(R = \text{H} \) \(^{11}\)C]ketoprofen) was selected for further investigation because of its favourable affinity and selectivity for MAO-B. High specific binding to regions with high MAO-B activity was observed in post-mortem human...
brain autoradiography and in vivo PET studies in a cynomolgus monkey demonstrated high brain uptake and accumulation in known MAO-B rich regions [186].

F. P2X7 Receptor (P2X7R)

The P2X7R appears to play an important role in cerebral inflammation and neurodegeneration and could be an interesting target for visualization of neuroinflammatory processes. The P2X7R selectively expressed on cells of hematopoietic lineage including mast cells, lymphocytes, erythrocytes, fibroblasts, peripheral macrophages, epidermal Langerhans cells and within the nervous system on microglia, belongs to the family of purinergic ATP (adenosine-5'-triphosphate) binding receptors and has been reported to play a main role in cell-to-cell communication, cell proliferation, cytokine release and cell death [187,188]. These purinergic P2 receptors are categorized into two major families: the P2X ionotropic receptors (ATP-gated ion channels) and the P2Y metabotropic receptors (G protein-coupled receptors). In response to extracellular ATP, P2X7R functions as an ion channel and is permeable to small cations such as Ca2+, K+ and Na+. Prolonged activation of P2X7R causes formation of a reversible plasma membrane pore permeable to molecules as large as 900 Da [188-190]. ATP is the only known endogenous activator of P2X7R. Under physiological circumstances, extracellular ATP concentrations are low. On the contrary, intracellular ATP concentrations are in the millimolar range. Activated immune cells, macrophages, microglia, platelets and dying cells may release high amounts of ATP into the pericellular space, explaining the high extracellular ATP concentration during inflammation. In addition, proinflammatory cytokines induce upregulation of P2X7R expression and increase its sensitivity to ATP [188]. Upregulation of P2X7R has been demonstrated in several animal models such as the AD, HD and a prion disease animal model. More specifically for the AD model, Aβ triggers ATP release, increases intracellular Ca2+, IL-1β secretion and generation of superoxide and plasma membrane permeabilization in microglia from WT but not in P2X7R-deleted mice [189,191,192]. Recently, Diaz-Hernandez et al. [193] demonstrated that in vivo P2X7R inhibition reduces Aβ plaques in AD through GSK3β and secretases [193]. This suggests that P2X7R upregulation is correlated with activation of microglia and thus with the inflammatory response leading to progression of neuronal loss in AD. Suppression of the receptor attenuates the toxic effects of P2X7R activation and plays a key role in pain modulation [188,194]. These facts make P2X7R an interesting therapeutic target as well as a biomarker for neuroinflammation and for neurodegenerative disorders [189].

A number of P2X7R antagonists including KN-62, PPADS, oxidized ATP and Brilliant Blue G have been developed in the past decade [195]. These antagonists however have a differential affinity for the human versus rodent P2X7R limiting their usefulness as an in vivo tool to examine the P2X7R expression in rat models of neuroinflammation. Recently, novel series of P2X7R antagonists have been introduced: adamantane amides (f.e. A-847227 and GSK314181A), cubyl amides, substituted tri/tetrazoles (f.e. A-438079) and cyanoguanidines (f.e. A-740003 and A-804598) (Fig. 6) [190,194,196-203]. Compared to the previous antagonists, these novel series show enhanced potency and selectivity as antagonists at both the rat and the human P2X7R [195,204,205]. Besides the antagonists mentioned above, several other scaffolds have been
used and optimized in structure-activity relationship (SAR) studies. Beswick et al. [206] developed a novel series of (1H-pyrazol-4-yl)acetamide P2X7 antagonists with good in vitro potency, selectivity and pharmacokinetic properties [206]. Several other SAR studies have been performed by GlaxoSmithKline [207-209]. Also Pfizer, Merck Research Laboratories, Janssen Research and Development and other research groups performed SAR studies [210-217]. Comprehensive reviews on P2X7 antagonists and their therapeutic efficacy/use have been published by Donnelly-Roberts et al. [218], Gunosewoyo and Kassiou [219]. At this moment, no validated PET tracer to visualize P2X7R upregulation has been reported. Therefore it could be interesting to radiolabel these high-affinity P2X7R-antagonists and evaluate them as potential neuroinflammation markers.

G. Histamine 4 Receptor (H4R)

Histamine (Fig. 7) is an endogenous short-acting amine formed from the basic amino acid histidine. Histamine exerts a wide range of biological effects through binding to four G protein-coupled receptors (GPCRs) and is known as a mediator in several (patho)physiological conditions. Unfortunately, less is known about its function in the brain. H1 and H2 receptors (H1R and H2R) are widely distributed and their function includes immunomodulation and gastric acid secretion, respectively [220,221]. The H3 receptor (H3R) is predominantly expressed in neurons and modulates neurotransmission [222,223]. The H4R is the most recently discovered histamine receptor. It is prominently expressed in cells and tissues of the immune system and modulates the immune system [224,225]. All four GPCRs have been subject of intensive research and drugs acting on the histaminergic system have been developed. H1R and H2R antagonists are used to treat allergy and gastrointestinal disorders, respectively. H3R antagonists could be used in treatment of dementias, obesity psychotic and sleep disorders. Nowadays, therapeutic exploitation of the H4R in inflammation and cancer is of great interest [225]. The immunomodulation in cytokine production and the presence of H4R in the immune system imply that the H4R is involved in inflammatory processes as asthma, allergic disorders, autoimmune diseases and thus could be an interesting imaging target to visualize neuroinflammation. Interesting lead structures to synthesise H4R PET ligands can be found in literature. Leurs et al. [224] describe several H4R ligands with nanomolar affinity such as JNJ7777120, VUF10558 and A-943931 (Fig. 7). These molecules could be interesting to be labeled with carbon-11 or fluorine-18 [224]. Other H4R ligands have been developed by Strakhova et al. [226], Sander et al. [227], Savall et al. [228], Mowbray et al. [229] and Lane et al. [230] and are also interesting for labeling with carbon-11 or fluorine-18. Two of the mentioned lead structures, JNJ7777120 and VUF10558, were radiolabeled with carbon-11 and currently, [11C]JNJ7777120 is being evaluated in vivo [231].

3. CONCLUSION

As the incidence of neurodegenerative disorders increases and treatment is in most cases only effective in the early stage of disease, diagnosis needs to be as early as possible. Since these neuropathologies are accompanied with neuroinflammation, detection of neuroinflammation is an interesting target for diagnosis and follow-up of disease progression and treatment.

Various targets are upregulated during neuroinflammatory processes and in neuropathologies. For each of these inflammation related targets, several PET tracers have been developed, evaluated and optimized. However, selecting the most reliable target to image neuroinflammation is difficult since interspecies differences in expression levels and protein sequence exist. In addition, the various animal disease models used to evaluate these tracers display different expression patterns of the upregulated proteins. It would be interesting to know which target in which disease is most prominently upregulated, this in order to correctly evaluate the degree of cerebral inflammation. Furthermore, since the activated microglia cells overexpress several inflammation related proteins, it is difficult to study the specificity of the tracer binding in these general inflammation models. Blocking all other known upregulated binding sites is necessary to determine the specificity. Development of
animal models with local overexpression of a specific neuroinflammatory target protein would be more straightforward. In case large interspecies differences with regard to affinity of ligands exists, the animal models should preferably overexpress human target proteins. Another important issue to bear in mind is the possible damage to the BBB in certain animal models. Before concluding there is an increased expression of the inflammation related target, it should be verified whether BBB integrity is not affected (for example with magnetic resonance imaging).

Up till now TSPO is the most studied target to image neuroinflammation. However, recent findings suggest a TSPO polymorphism resulting in differences in binding affinity of PET radioligands for TSPO. As a consequence, knowledge of the binding status of the individual patient is required to correctly interpret the PET images.

The development of radiotracers for imaging TSPO, CB2 and COX continues but recently also new interesting targets have appeared. This, together with the search for better animal models of neuroinflammation makes the search for an ideal biomarker of neuroinflammation continuous and fascinating.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

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