EVALUATION OF CEREBRAL NEUROTRANSMITTER PHYSIOLOGY AND PATHOPHYSIOLOGY WITH PET AND SPET IMAGING MODALITIES IN ANIMAL MODELS

Onderzoek van neurotransmissiefysiologie en pathologie in de hersenen met behulp van PET- en SPET-technieken in proefdiermodellen

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ABSTRACT

Since "positron emission tomography" (PET) and "single photon emission tomography" (SPET) have become widely available as imaging modalities, they have been used to perform studies on neuroreceptor physiology and pathophysiology in man and animals. Several neurological and psychiatric diseases related to neurotransmitters and receptor functioning have been investigated. In order to investigate the biological effects of new compounds, this imaging modality is becoming more and more popular in the pharmaceutical industry, since pharmacokinetics and pharmacodynamics of new drugs can now be evaluated in vivo, omitting extensive in vitro examinations. The response to pharmaceuticals can thus be evaluated more objectively than with the more subjective assessment of patient condition based on clinical examination and the interpretation of neuropsychological tests. This review focuses on the technology of this imaging modality and on the nature of the radioligands used for neuroreceptor imaging. A brief overview will be given of the research on neuroreceptor physiology, neuropathology, drug evaluation and substance abuse, with the emphasis on animal models. Future prospects for implementation in veterinary medicine are discussed.

SAMENVATTING

Sinds "positron emission tomography" (PET) en "single photon emission tomography" als beeldvormende technieken meer algemeen toegankelijk zijn geworden, is het onderzoek van de neuroreceptor fysiologie en pathofysiologie op gang gekomen bij zowel mens als dier. Verschillende neurologische en psychiatrische afwijkingen waarbij neurotransmitters en receptor functie betrokken zijn, werden reeds in beeld gebracht. Ook de farmaceutische industrie vertoont interesse voor dit onderzoek omwille van het feit dat de farmacokinetiek en -dynamiek van chemische substanties *in vivo* kunnen bestudeerd worden. Daarbij wordt het ontwikkelingsproces van nieuwe medicamenten versneld. Het resultaat van de medicatie kan objectief gevolgd worden met deze techniek als aanvulling op de klinische evaluatie van de patiënt en van de interpretatie van neuropsychologische testen, die beide een zekere subjectiviteit inhouden.

Dit overzicht gaat voornamelijk over het technisch aspect van deze beeldvorming en de eigenschappen van de radioliganden die hiervoor in aanmerking komen. Verder wordt een stand van zaken gegeven betreffende de toepassing van het neuroreceptor onderzoek met nadruk op diermodellen die hiervoor gebruikt worden. Tenslotte wordt het mogelijk gebruik in de diergeneeskunde geëvalueerd.

INTRODUCTION

In the 19th century, Langley formulated the concept of "receptive substances" which involves the interaction of drugs with tissue. Later, the Nobel prize winner Ehrlich proposed that interaction with toxic substances is caused by the presence of certain cell surface groups (receptors) showing binding properties with these toxins (ligands). This binding was due to high complementarity between the stereochemical configuration of the ligand and the receptor with which it interacts. With the formulation of the receptor concept, an explanation was found for the specific effects of tracer amounts of substrate on a target organ.

Classification of receptors is based on their chemical specificity (e.g. the α- and β- adrenergic receptors) or on their anatomical localization (e.g. pre- and postsynaptic receptors, intra-and extracellular receptors), while ligands are classified according to their ability to generate a response. Full agonists will generate a maximal receptor response, while partial agonists will never produce a maximal activation of the receptor, no matter how high a concentration is applied. Whether a ligand is a full or a partial agonist is a tissue- and drug- dependent phenomenon, although endogenous agonists for a receptor type usually behave as full agonists. Antagonists block the response in a reversible, irreversible, competitive, non-competitive or mixed way. Distribution and kinetics of the ligand (drug or endogenous substrate) can be quantified with *in vitro*, *ex vivo* (injection of the ligand *in vivo*, measuring tissue activity after death) and *in vivo* imaging techniques using radioactive labeled molecules. I*n vitro* techniques have been used to evaluate ligand-receptor responses and to quantify receptors. In recent years, functional imaging techniques with positron emission tomography (PET) and single photon emission tomography (SPET) are gradually gaining importance because they enable ligand-receptor interactions to be evaluated in their natural environment in the living subject.

This technology is now more commonly being applied in drug development. Functional imaging is also being used as a research and diagnostic tool for studying neurologic and neuropsychiatric diseases in humans, thus providing insight into complex receptor interactions. In this context, animal models were introduced to investigate neuropathologic disorders, usually created by genetic, surgical, pharmacological or behavioral manipulation of the animal. In most cases, rodents and non-human primates have been used, although it has been suggested that dogs would provide a valid alternative model because they are widely available, rather cheap and easy to keep and handle, compared to non-human primates. It has been proposed that dogs develop analogue conditions to some of the human psychiatric disorders and may therefore serve as a natural model for human disease. This has lead to the investigation of natural canine models with behavioral and neuropathological similarities to human disease. In this context, canine cognitive dysfunction, dominance aggression and impulsivity, and compulsive disorders have been reported to show clinical and/or neuropathological resemblance to human diseases such as dementia, personality disorders and obsessive compulsive disorder, respectively. From a veterinary point of view, this technique may facilitate research on the mechanisms of neurological and behavioral disorders in dogs, and it may also be used to monitor the effects of therapy.

The aim of this article is to give a review of receptor imaging techniques and their implementation, with emphasis on the *in vivo* imaging of neuroreceptors with PET and SPET modalities. Special attention will be given to neuroreceptor research in which animal models are used to illustrate the clinical potential of this modality for investigating the pathogenesis and treatment of behavioral diseases in man and animals.

METHODOLOGICAL CONSIDERATIONS

Nuclear medicine imaging methods

Receptor imaging in vitro

Neurotransmitter receptors can be visualized and quantified in frozen tissue sections using autoradiography. In order to achieve high spatial resolution, radionuclides with low β· emission energies, such as ^{3}H, ^{125}I and ^{14}C, are used. These radionuclides are bound to highly specific probes, thus providing a radiolabeled ligand (e.g. ^{3}H-spiperone). These examinations can be performed either *in vitro* or *ex vivo*. Specific binding, which is binding of the radiolabeled ligand with the receptor, and non-specific binding, which is the activity persisting in the presence of an excess of a specific nonradioactive displacer, can be quantified, thus providing an estimation of receptor density (B_{max}) and affinity ($1/K_D$) (K_D= dissociation constant at equilibrum). Another method for evaluating the effects of receptor binding is the measurement of the cellular responses elicited by the ligand-receptor interaction. Changes in cyclic adenosine monophosphate (cAMP) concentration, glucose production or electrical and mechanical responses enable biochemical characterization of the receptor.

Since the natural environment of pre- and postsynaptic membranes is not accounted for *in vitro*, assumptions concerning drug interaction or ligand-receptor interaction from *in vitro* experiments should be regarded with caution when adopted to receptor estimation *in vivo*. *Ex vivo* techniques require the death of the animal under investigation, since the β^- particles, which are emitted during ^{3}H, ^{14}C or ^{125}I decay, are strongly attenuated by tissue and therefore cannot be measured with external detecting devices.

Immunohistochemistry offers an alternative method for visualizing receptors *in vitro*. This technique is not based on nuclear medicine isotopes, however, and therefore falls outside the scope of this article.

Receptor imaging in vivo

In vivo techniques offer a more correct representation of receptor expression, because the receptor characteristics can be altered as a result of the interaction with its intra- and extracellular regulatory mechanisms. The PET and SPET functional imaging modalities enable imaging of the receptor with γ and β^+ emitting radioisotopes incorporated into ligands, thus permitting evaluation of biological processes *in vivo* in a safe and non-invasive manner (Table 1). Due to the high affinity and specificity of the radioligand for the receptor and because of the sensitivity of the imaging system, only trace amounts - usually in the picomolar range - of this radiolabelled ligand are applied, thus avoiding physiological or toxic effects. *In vivo,* sequential studies are possible and time/activity curves in regions of interest can be generated.

SPET imaging (Fig 1a)

SPET is based on the emission of photons that are registered by external detectors, the so-called gamma cameras. It is mostly technetium (^{99m}Tc, $T_{½}$=6h) or iodine (^{123}I, $T_{½}$=13h) that are used as radionuclides. The longer half-lives of these radionuclides preclude repetitive investigations on the same individual on the same day, but they can be an advantage for studies on receptor occupancy requiring prolonged scanning times. Spatial resolution depends on the collimator and camera configuration, but in general it is lower than with PET (7-10 mm vs 3-4 mm). In order to ensure that the photons are deriving from a particular volume of interest, lead shielding (collimators) is used to limit detection of scattered photons. This yields a significant loss of signal and corresponding loss of sensitivity compared to PET. Another important limitation is that, due to attenuation, scatter and partial volume effects, registered photons are not with certainty derived from the actual region of interest. This precludes absolute quantification methods (Table 1). One important advantage of SPET is that the radionuclides used are commercially available and no cyclotron is needed at the imaging facility.

PET imaging (Fig. 1b)

Positron emitting radionuclides are used in PET studies. The most commonly used radionuclides are ^{15}O ($T_{½}$=2 min), ^{13}N ($T_{½}$= 10 min), ^{11}C ($T_{½}$=20 min), ^{18}F ($T_{½}$=110 min) and ^{76}Br ($T_{½}$=16 h). The short half-lives of ^{11}C and ^{18}F enable repetitive investigations

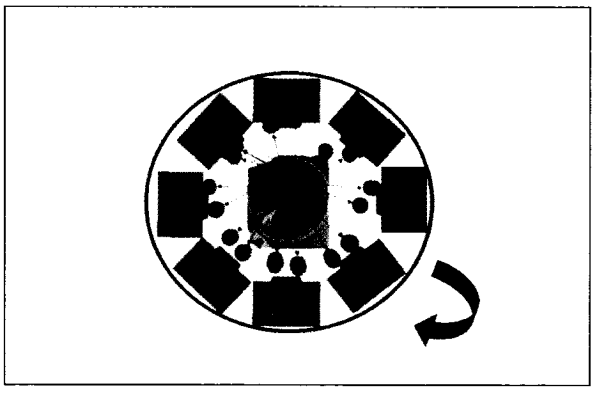

Fig. 1a. The SPET acquisition mode consists of a computer assisted rotation of one or multiple detector heads, which are equipped with dedicated collimators and which rotate in a 360° circular or elliptical path around the patient, focussing on the target organ and registering every photon emitted (represented by the red dots).

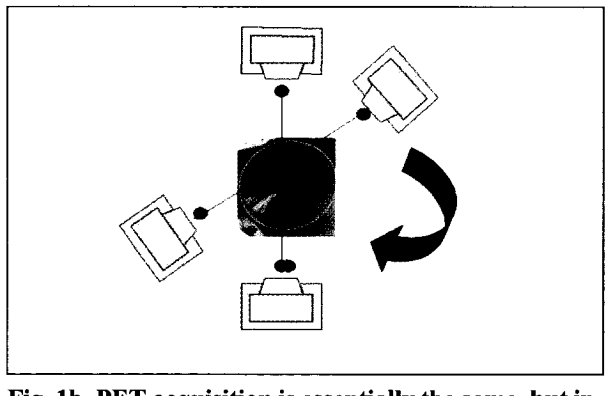

Fig. 1b. PET acquisition is essentially the same, but involves the registration of two coincident photons (each with energy of 511 KeV), which were generated through the annihilation of a single positron and emitted at an angle of 180°. These two photons are detected simultaneously by the crystal and, as such, are registered as having been emitted from the same source in the organ. This has the advantage that the spatial registration of an event is more accurate than with SPET. An incident photon without coincidence of the other photon at 180° will not be registered as a true event (interrupted arrow in yellow).

Table 1. Characteristics of commonly used radionuclides and spatial resolution for PET and SPET imaging modalities.

	SPET (Fig. 1a)	PET (Fig. 1b)
Characteristics isotopes	Gamma photons electromagnetic radiation Emitted photon energy depending on the isotopes ^{99m}Tc=140 keV; ^{123}I=164keV	Beta+ particle radiation. Emitted photon energy: 2x 511 keV
Commonly used isotopes T1/2	^{99m}Tc 6h; ^{123}I 13h	^{18}F 110 min, ^{15}O 2min; ^{13}N 10 min; ^{11}C 20 min; ^{76}Br 16h
Spatial resolution	7-8mm	3-4mm

within a short time interval in the same individual, using the same or different radioligands under identical or different test conditions. Following emission, these positrons travel a few millimeters in matter before they interact with an electron, producing annihilation energy in the form of two opposite photons with energy of 511 keV each. Measurements depend on the simultaneous detection by external detectors of these photon pairs, which are emitted at 180° to each other. One disadvantage is that the commonly used radionuclides have a short half-life and must be generated by a cyclotron in the immediate vicinity of the camera, for which reason, an on-site chemistry facility is required for synthesizing the radioligand (Table1).

Radioligand properties

Radionuclides as a matter of concern

Labeling of the ligand can alter its biological activity, rendering it unsuitable for binding with the receptor. Labeling with ^{11}C, which is found in all organic compounds, usually does not cause alteration of the physicochemical properties of the receptor ligand and thus ^{11}C labeling opens the door to the labeling of a wide variety of ligands. Labeling with ^{18}F or ^{76}Br, which are not generally found in natural compounds, may alter the ligand's properties. For the same reason, labeling with either ^{99m}Tc or ^{123}I usually has a significant impact on the physicochemical properties of the ligand and, what is more, these isotopes are large molecules, a fact which may potentially lead to steric hindrance. The duration of the labeling procedure is another important issue because of the short half-life of some of the isotopes. Therefore, especially with regard to the short half-life of commonly used PET tracers, this procedure should be kept as short as possible and the label should be introduced as late as possible in the synthetic procedure.

Ligands as matter of concern

Exogenous antagonists are preferred ligands because dilution with endogenous agonists is less likely to happen, affinity for the receptor is usually higher and receptor responses interfering with the binding estimation are less likely to occur.

Candidate neuroreceptor radioligands need to cross the blood-brain barrier, which entails an amount of lipophilicity of the ligand. On the other hand, lipophilicity increases undesired binding to non-specific molecules (proteins, blood cells, etc.). Lipophilicity, the value of the octanol-water partition coefficient, is expressed in log units (log P) and, depending on the methods used for measuring log P, the values should be between 1 and 3.5 for neuroimaging tracers in order to be clinically useful.

Furthermore, tracer metabolism *in vivo* may result in both labeled and non-labeled metabolites. When crossing the blood-brain barrier, labeled metabolites will increase background radioactivity by binding to non-specific molecules. Therefore, ideally, metabolism should be away from the brain, the metabolites should not be labeled and, if they are, they should not cross the blood-brain barrier.

Another important issue in the evaluation of the suitability of a tracer for receptor imaging is the affinity and the selectivity of the radioligand. Affinity is

the chemical strength of the binding, which is defined as the ratio of association and dissociation, while selectivity refers to the strength of the ligand interaction with one receptor as compared to interactions with other receptors (the ability to recognize target receptor molecules). Biological substrates frequently lack selectivity and high affinity. High affinity would be a negative quality for endogenous neurotransmitters since neuroreceptors need to have quick, short reactions when activated by a stimulus. The opposite is true for receptor probes used in research, which must have high affinity and selectivity to permit imaging.

A final important methodological issue is the quantification of receptor binding. PET and SPET data can be analyzed at three levels. The simplest approach is visual inspection, determining areas of increased or decreased binding. This method is of limited value since it is subjective and biased by the skills of the interpreter. The next level of data analysis is the semi-quantitative or "reference region" approach, which is based on the condition that the binding with the receptor is reversible and that a steady state between uptake and washout of the radioligand after a single bolus injection can be achieved. Receptor density is then calculated as the ratio of binding in regions with high density of receptors (specific binding) to that in low density areas (non-specific binding) which represent the reference region. The main advantage of this method is that it provides a simple and quick semi-quantification procedure for clinical use. At the highest level, sophisticated mathematical procedures based on compartmental modeling have been developed to calculate K_D and B_{max} more accurately. Most of these procedures are elaborate, requiring arterial sampling, and are therefore not very workable in clinical settings.

Table 2 provides an overview of radioligands, which are classified per neurotransmitter system and are tested *in vivo* with PET and SPET modalities in animals (mostly in primates).

APPLICATIONS

Two major lines of neuroimaging research using animal models will be discussed and evaluated: first, the influence on neuroreceptors of exogenous substances, which are used in the pharmaceutical industry and in substance abuse disorders; secondly, neuropsychiatric disorders involving neuroreceptors.

Exogenous substances

Drug industry

PET and SPET imaging modalities have become a valuable tool in the development of new drugs. The advantage of functional imaging is that the doses of the labeled compounds are so low that pharmacological and toxicological effects involve no risk to the individual. For this reason, information on distribution and receptor binding can be obtained very early in the development of the drug. Two main strategies are followed in evaluating the pharmacological receptor occupancy. First, *direct studies* in which the drug itself is labeled and, second, *indirect studies* in which the direct or indirect effects of the drug on a target receptor are investigated by the use of receptor-specific radioligands. Receptor occupancy studies can be used to determine the dose at which drugs are therapeutically effective and/or have the slightest side effects.

Substance abuse

The so-called 'challenging' studies can provide insight into the pathophysiological changes caused by abusive substances at the receptor level. Some drugs will induce activation or inhibition of a particular neurotransmitter system, resulting in the facilitation or inhibition of neurotransmitter release, which is then translated into altered binding of the radioligand to the receptor. This has proved useful in the evaluation of the toxic effects of frequently abused drugs such as ketamine ("Special K"), amphetamines ("speed"), cocaine, phencyclidine (PCP) ("Angel Dust") and 3,4-methylenedioxymethamphetamine (MDMA) ("Ecstacy"). Most drugs involved in drug abuse increase the levels of extracellular mesolimbic dopamine (DA) (the "pleasure" or "reward" neurotransmitter) as the final common pathway (albeit through different mechanisms), a fact which explains their reinforcing properties. As an example, acute administration of ketamine or PCP, both competitive antagonists of the NMDA receptor, has an impact on radioligand binding both to the dopamine-2 (D2) receptor and to the dopamine transporter receptor (DAT), due to the acute release of competing endogenous dopamine. Along this line, the influence of acute administration of various psychostimulating drugs on endogenous synaptic DA has been demonstrated in primate studies. The wash-out rates of radioligands for D2 and the dopamine transporter (DAT) (which is responsible for the re-uptake of synaptic dopamine into the pre-synaptic neuron) were compared and the diffe-

Table 2. Survey of radioligands tested in animal models. The radioligands marked in bold are currently used in the USA in clinical human studies.

Neurotransmitter system	Radioligand PET	SPET
Dopamine 1	[^{11}C]**SCH 23390** [Halldin *et al.*,1986; Ekesbo *et al.*, 1999] [^{11}C]SCH 39166 [Sedvall *et al.*, 1991] [^{18}**F]SCH 38548** [Mukherjee *et al.*, 1993; Yang *et al.*,1996] [^{11}C]NNC 22-0010, 22-2015 [Foged *et al.*, 1996b; Foged *et al.*,1998] [^{11}**C]NNC 112**, 687, 756 [Karlsson *et al.*, 1993; Halldin *et al.*,1993; Halldin *et al.*, 1998]	
Dopamine 2	[^{11}C]NMB [Suehiro *et al.*,1990] [^{18}F]NMB [Moerlein *et al.*,1997] [^{11}**C]raclopride** [Dewey *et al.*,1993] [^{11}C]etclopride [Halldin *et al.*,1990] [^{11}**C]FLB** [Halldin *et al.*,1995] [^{11}**C]NMSP** [Kobasashi *et al.*,1995] [^{18}**F]5-FprEpid** [Kessler *et al.*,1993b] [^{18}F]FEB [Moerlein, Perlmutter, 1992b] [^{18}**F]NCQ-115** [Halldin *et al.*,1994] [^{18}F]fallypride [Mukherjee *et al.*,1996; [Mukherjee *et al.*,2001] [^{18}**F]fluoroethylspiperone** [Welch *et al.*, 1988; Coenen *et al.*,1988; Bahn *et al.*, 1989; Jovkar *et al.*, 1990] [^{18}**F]FCP** [Mach *et al.*,1996] [^{11}**C]FLB 457**, 463 [Loc'h *et al.*, 1996; Delforge *et al.*, 2003]	[^{123}**I]epidepride** [Kessler *et al.*, 1993a; Almeida *et al.*, 1999] [^{123}**I]IBZM** [Kung *et al.*, 1989; Al-Tikriti *et al.*,1994; Laruelle *et al.*, 1994b] [^{123}I]IBF Billings *et al.*, 1993; Al-Tikriti *et al.*, 1994; Laruelle *et al.*, 1997] [^{123}I]NCQ 298 [Hall *et al.*, 1991]
Dopamine transporter DAT	[^{11}**C]cocaine** [Gatley *et al.*, 1994] [^{18}**F]fluorococaine** [Wilson *et al.*, 1996] [^{11}**C]β-CITFP** [Lundkvist *et al.*, 1995; [Lundkvist *et al.*, 1997] [^{11}**C]β-CIT** [Muller *et al.*, 1993]	[^{123}I]IPT [Malison *et al.*, 1995] [^{123}I] β-CITFE, -CITFP [Baldwin *et al.*, 1995] [^{123}I] β-CIT [Laruelle *et al.*, 1993; Scanley *et al.*, 2000]

	[^{11}C]altoprane [Madras *et al.*,1998; Fischman *et al.*,2001] **[^{11}C]methylphenidate** [Gatley *et al.*, 1995] [^{11}C]β-CITFE [Halldin *et al.*, 1996] **[^{11}C]WIN 35428** =beta CFT [Wong *et al.*, 1993; Melega *et al.*, 2000] [^{11}C]PE 21 [Poyot *et al.*,2001] **[^{18}F]FECNT** [Goodman *et al.*,2000] [^{18}F]GBR 13119 [Kilbourn *et al.*, 1989] [^{76}Br]PE2Br [Helfenbein *et al.*,1999a]	[^{99m}Tc]TRODAT [Dresel *et al.*, 1999; Acton *et al.*, 1999b]
Serotonin-1A	[^{11}C]**WAY-100635** [Mathis *et al.*, 1994; Osman *et al.*, 1996; Farde *et al.*, 1997; Andree *et al.*, 2000] [^{11}C]DWAY [Pike *et al.*, 1998] [^{11}C]NAD-299 [Sandell *et al.*, 2000] **[^{18}F]FCWAY** [Carson *et al.*, 2000] [^{18}F]*p*-MPPF [Shiue *et al.*, 1997]	
Serotonin-2A	**[^{11}C]MDL 100907** [Lundkvist *et al.*, 1996] [^{11}C]MBL [Wong *et al.*, 1987] **[^{18}F]altanserin** [Soares *et al.*, 2001] [^{18}F]deuteroaltanserin [Staley *et al.*, 2001; Soares *et al.*, 2001] **[^{18}F]setoperone** [Blin *et al.*, 1988]	[^{123}I]5-I-R91150 [Abi-Dargham *et al.*, 1997; Peremans *et al.*, 2002]
Serotonin transporter SERT	[^{11}C]DASB [Szabo *et al.*, 2002] **[^{11}C]McN5652** [Szabo *et al.*, 2002] [^{11}C]nor-β-CIT [Bergstrom *et al.*, 1997] [^{11}C]RTI-357 [Helfenbein *et al.*, 1999b] [^{11}C]MADAM [Halldin *et al.*, 2002]	[^{123}I]β-CIT [Baldwin *et al.*, 1993; Laruelle *et al.*, 1993] [^{123}I]5-I-6-NQP [Jagust *et al.*, 1993; Jagust *et al.*, 1996] [^{123}I]ADAM [Acton *et al.*, 2001] [^{123}I]ODAM [Acton *et al.*, 1999c] [^{123}I]IDAM [Acton *et al.*, 1999a]
Histamine	[^{11}C]Pyrilamine [Villemagne *et al.*, 1991]	

Opiate	[^{11}C] buprenorphine [Shine *et al.*, 1991; Galynker *et al.*, 1996]	[^{123}I]-O-IA-DPN [Lever *et al.*, 1998]
GABA/benzodiazepine	[^{11}C]flumazenil =Ro 15-1788 [Brouillet *et al.*, 1990; Moerlein, Perlmutter, 1992a; Inoue *et al.*, 2001] [^{11}C]Ro15-4513 [Halldin *et al.*, 1992; Lingford-Hughes *et al.*, 2002] [^{76}Br] NNC 13-8199 [Foged *et al.*, 1997]	[^{123}I]iomazenil =Ro16 0154 [Sybirska *et al.*, 1993; Laruelle *et al.*, 1994a; Laruelle *et al.*, 1994c] [^{123}I]NNC 13-8241 [Foged *et al.*, 1996a]
Muscarinic receptors	[^{11}C]NMBP [Skaddan *et al.*, 2003] [^{11}C]3-MPB [Tsukada *et al.*, 2001d] [^{11}C]benztropin [Dewey *et al.*, 1990] [^{18}F]FP-TZTP [Carson *et al.*, 1998] [^{11}C]benzilate derivatives [Varastet *et al.*, 1992; Mulholland *et al.*, 1992] [^{76}Br]benzilates [Strijckmans *et al.*, 1997]	[^{123}I]IQNP [Bergstrom *et al.*, 1999; Nobuhara *et al.*, 2000]
Nicotinic receptors	[^{18}F]2-F-A-85380 [Valette *et al.*, 1999] [^{18}F]6-I-A-85380 [Valette *et al.*, 1999] [^{11}C]MPA [Sihver *et al.*, 1999]	[^{123}I]5-I-85380 [Chefer *et al.*, 1998; Musachio *et al.*, 1999][Fujita *et al.*, 2000]

rence was found to be consistent with the dose-dependent ability of each drug to elevate synaptic dopamine levels. The effects of chronic abuse can be evaluated, as was demonstrated in primate studies following chronic amphetamine intake. Decreased binding of DAT and D2 radioligands was found, which is suggestive of decreased functioning and down-regulation of the receptors. Concerning the neurochemical effects of MDMA, reduction in radioligand binding of the 5-HT2A receptor and the serotonin transporter (SERT) has been demonstrated both in man and animals, suggesting serotonergic neurotoxicity. Research has not been limited to illegal drugs, but has also provided interesting information concerning addictive substances such as nicotine and benzodiazepines. Although chronic nicotine administration, at a dose equivalent to 20 cigarettes per day, resulted in an upregulation of neuronal acetylcholine (AcCh) receptors *in vivo* with a SPET radioligand in baboons, the effect of nicotine on the dopaminergic system was shown to be insufficient to displace [^{11}C]raclopride in monkey brain. The inhibitory affects of gamma-aminobutyric acid (GABA) neurons on endogenous dopamine concentrations, following the administration of a clinically prescribed benzodiazepine agonist, have been demonstrated in primates with a radioligand for D2 receptors.

Characterization of neuropsychiatric disorders

Dysregulation of one or more neurotransmitter systems is implicated in the pathophysiology of many neuropsychiatric disorders, such as Parkinson's disease, schizophrenia and mood disorders. Since animal models are used more frequently in research on human neuropsychiatric disorders and their treatment, functional imaging of the animal brain is becoming increasingly important.

In the following review, only studies involving the use of animal models and functional imaging with PET or SPET modalities will be covered.

Ischemia

Brain ischemia resulting from thrombotic or embolitic processes is becoming an increasing problem in the elderly population. Since ischemia is accompanied by loss of synapses and neurons, neuroreceptor imaging could possibly be an indirect method for evaluating the extent of the insult and monitor revalidation. An animal model using experimentally induced hypoxic ischemia through unilateral arteria cerebri media occlusion in rats and primates showed a significant reduction in binding potential of the central benzodiazepine receptor, the muscarinic cholinergic receptors and the D2 receptors in the absence of gross anatomical alterations. The radioligand distribution correlated well with the *in vitro* neuronal and synaptic distributions. This may have both diagnostic and therapeutic implications concerning early ischemic neuron degeneration and synaptic damage.

Neurodegenerative disorders

Neurodegenerative disorders are characterized by a progressive degeneration of neurons and neurotransmitter systems in predilected brain regions, leading to functional symptoms associated with these brain regions.The most common form of dementia in humans is Alzheimer's disease (AD), a disorder characterized by a reduction in brain acetylcholine content in frontal and temporoparietal regions and, histologically, by excessive interstitional and perivascular deposition of amyloid and intracellular accumulation of neurofibrillar tangles. Clinical differentiation from other dementia syndromes, such as frontal lobe or subcortial dementia, is not always straightforward due to the numerous overlapping symptoms and inconclusive morphological changes which complicate the categorization into irreversible organic pathology and treatable conditions. PET and SPET (perfusion, metabolism and neuroreceptor imaging) can contribute significantly in questionable situations by enabling the localization of functional deficits, thereby enhancing diagnostic accuracy. Studies with the PET ligand ^{11}C (+)3-MPB in monkeys demonstrated an age-related reduction of cholinergic receptor density in frontal and temporal cortices, the regions affected initially in Alzheimer's disease patients. Moreover, this imaging modality may offer important information on pharmacotherapeutic strategies with acetylcholine-esterase inhibitors in the treatment of AD.

Another important neurodegenerative disorder is Parkinson's disease (PD), an extrapyramidal movement disorder which is associated with degeneration of dopamine-containing neurones in the nigrostriatal pathway. Since no natural animal models are available, a parkinsonian model for the disease is created by the injection of 1-methyl-4-1,2,3,6-tetrahydropyridine (MPTP), a toxic substance that destroys the dopaminergic neurones in the substantia nigra. Radioligand studies in primates showed increased D2 radioligand binding, suggesting reduced presynaptic dopamine availability and/or upregulation of the receptor similar to findings in patients with Parkinson's disease. In addition, decreased DAT radioligand binding was also present in these primates, being demonstrated with PET and SPET tracers, and there was found to be a correlation between DAT density and clinical staging of PD.

Schizophrenia

Alterations in neurotransmitter biochemistry have been examined extensively in schizophrenia, a disease that has been associated with a dysfunctional dopaminergic and serotonergic system. The pathogenesis of this disease has not been completely elucidated and both developmental and degenerative etiologic components have been put forward. Schizophrenia is characterized by the occurrence of both positive (psychosis with hallucinations, delusions and paranoia) and negative (psychosocial retardation, lack of motivation) symptoms, similar to those encountered in drug abuse. Since animals lack the ability to speak, a fact which precludes any accurate assessment of psychotic symptoms and cognitive dysfunction, natural animal models are not available. This state of affairs limits research into 'partial' animal models based mainly on the use of psychotomimetic drugs such as ketamine and amphetamine. Most animal research, however, has focused on the evaluation of therapy with neuroleptics and their side effects. The action mechanism of the conventional antipsychotic drugs (haloperidol, chlorpromazine, etc.) is based mainly on blockage of the D2 receptor, which alleviates the positive symptoms but exacerbates the extrapyramidal symptoms. Animal studies have shown that these extrapyramidal side effects are dose-dependent and that the degree of D2 receptor occupancy predicts the side effects. *In vivo* D2 receptor radioligand studies show that in over 70% of the cases, receptor binding produces an "anti-

psychotic" effect, corresponding in animal models to improvement of the "conditioned avoidance response", while greater than 80% occupancy induces extrapyramidal symptoms, which correspond in animal models to cataleptic symptoms.

Aggressive and impulsive behavior

One of the most replicated findings in biological psychiatry is the link between reduction of the serotonin metabolite 5-HIAA in the cerebrospinal fluid and impulsivity and aggression both in man and animals. Over years of study, it has become evident that dysfunctioning of the serotonergic system plays a crucial role in impulsive behavior and that this phenomenon can be influenced with serotonergic medication, both in man and in animals. A pilot study with a specific radioligand for the 5-HT2A receptor for SPET was set up to explore the contribution of the serotonergic system in canine impulsive aggression. A significant increase of cortical binding of this ligand was found in impulsive aggressive dogs as compared to dogs that did not show this behavior, thus suggesting involvement of the serotonergic system.

Epilepsy

Currently, epilepsy is thought to be caused by an imbalance between neuronal excitation (mostly via the NMDA system) and inhibition (mostly via the GABA system). A decreased GABA-benzodiazepine receptor level in epileptic foci compared to the contralateral reference region has been demonstrated in preliminary human and animal studies with radioligands. Animal studies have been set up to investigate the effect of potential anticonvulsant pharmacological probes on these receptors.

CONFOUNDING FACTORS

First, several studies in humans, in non-human primates and in rats have demonstrated that the binding of several neurotransmitter systems is influenced by age and gender and that modulation of receptor binding parameters can occur as a result of hormonal influences such as arise during the normal menstrual cycle and menopause.

Secondly, sedation and anesthesia are prerequisites for animal research on receptor imaging. The effects of several anesthetics are twofold. They can act on the receptor either directly or indirectly through other receptor systems and they can provoke circulatory disturbances, thus altering radioligand delivery to the brain. Therefore, it is important to select the anesthetic combination that provokes the least disturbances in the equilibrium achieved between the receptor and its radioligand and to stick to a single anesthetic protocol for a specific type of research.

Thirdly, recent studies concerning D2 receptor radioligand binding following pharmacological manipulations, which induce an increase or depletion of DA synaptic concentrations, have yielded conflicting results. Emerging data indicate that simple radioligand binding competition with the endogenous neurotransmitter is not the only mechanism involved. It has been hypothesized that receptor 'trafficking' (internalization of the receptor under agonistic influence) may play an important role. Paradoxical results from different radioligand studies may be the result of some radioligands passing the cellular membrane, which may result in binding to the intracellularly located receptor. This may in turn result in individual sensitivity to the intracellular environment and by consequence increase or decrease the affinity of the radioligand for the internalized receptor.

FUTURE PROSPECTS

Neurotransmitter receptor imaging may serve several purposes. First, it is used to study neuropsychiatric diseases in order to elucidate the pathophysiology of disease *in vivo* and to clarify clinical diagnostic and prognostic uncertainties. Instead of utilizing trial and error to see whether a drug might help, this imaging technique utilizes topographic localization in the brain to identify receptor abnormalities, thus providing guidance in the process of initiating and monitoring effective therapy. Due to the radiation burden, neither the examination of young people nor multiple examinations at different ages of the same individual can be carried out. Since the radiation burden is not a constraint in animal studies, repeated scanning of the same animal can be performed, starting from a very young age and extending on into senescence. This would provide important information on receptor development in the very early stages of life, before full maturation of the central nervous system.

From a veterinary point of view, the pathophysiology of behavioral problems, such as certain forms of canine aggression and impulsivity, and the effect of medical treatment, may be investigated. Moreover, changes in neurotransmitter systems appear to be involved in age related cognitive decline and in mood

disorders, which currently are important research topics not only in human, but also in veterinary medicine.

Neurotransmitter imaging plays an important role in monitoring neurologic and neuropsychiatric drug therapy. Pre- and post-therapy imaging can visualize the effects of drugs and can make a link with clinical progress or explain unresponsiveness to therapy. It is an important and indispensable tool for the pharmaceutical industry. It provides a means of evaluating *in vivo* the working mechanism of the drug under investigation, of estimating dosage, of monitoring side effects and of evaluating the therapeutic effects of newly developed drugs. Since serial scanning is not a problem in animal studies, drug-receptor interaction can also be monitored over time and related to clinical effects.

However, future research should also be directed towards elucidating pathophysiological receptor mechanisms. But, most probably not all subtypes of the receptor families are yet known; hence, interactions of the radioligand with a "known" receptor might not be as specific for that particular receptor. A second limiting factor is the availability of ligands for different receptors, since the stringent requirements of their design limits the variety of available probes. Concerning implementation in veterinary clinical medicine, radiopharmaceuticals will have to be available at affordable prices. Therefore, radiolabeled tracers for SPET will be very welcome, as will pharmaceuticals labeled with ^{99m}Tc.

In summary, neuroreceptor imaging provides important insight into the pathophysiology of diseases, thus resulting in more efficient treatment protocols. This technique may potentially be adopted by veterinary medicine when appropriate and affordable radiopharmaceuticals are available. It can be implemented in research on behavioral disorders in animals and, in particular, in the dog model. It can also be used to evaluate the kinetics and dynamics of therapeutic drugs and to monitor the outcome of therapies in clinical cases. Moreover, the use of larger animal models can be of relevance for understanding human cognitive, emotional and behavioral characteristics and deviations.

REFERENCES

The reference list can be obtained from the first author.