What to learn from in vivo opioidergic brain imaging?

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Abstract

Ligand-PET studies are attracting increasing interest in experimental and clinical research. As the most elaborated of PET techniques, ligand-PET allows the demonstration of receptor distributions, and thus, the delineation of neurochemical pathologies in the disease state. Recent developments are promising that ligand-PET will even allow to characterize dynamic and short-term changes in neurotransmission and will tremendously add to the understanding of neurophysiology on the receptor level. In pain studies, mainly the μ-opioidergic agonist [11C]-carfentanil and the unspecific opioid receptor antagonist [11C]-diprenorphine are applied. Utilizing these ligands the thalamus, prefrontal and cingulate cortex, basal ganglia and midbrain structures have been shown to possess high amounts of opioidergic receptors in vivo and it is well accepted, that the receptor density is higher in projections of the medial than those of the lateral pain system. Changes in receptor availability were observed in patients suffering from chronic pain. Rheumatoid arthritis, trigeminal neuralgia and central poststroke pain (CPSP) all lead to decreased ligand binding in pain processing regions during the painful period in comparison to pain free intervals or healthy subjects. These decreases may either be the consequence of increased endogenous release or indicate receptor internalization/down-regulation or loss of neurons carrying these receptors. Recent studies also evidenced [11C]-carfentanil binding changes due to acute experimental pain. One possible interpretation of these changes is that the PET-ligand might be displaced by endogenous opioidergic ligands. One major region, where this “ligand displacement” was observed, was the thalamus. These findings highlight the importance of the opioidergic system in pain processing and the power of ligand-PET to advance the understanding of pain.

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1. Introduction

With the development and improvement of new imaging techniques such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) over the last two decades, more and more cortical and subcortical structures involved in pain transmission have been revealed. Among these imaging methods, PET not only allows in vivo measurements of brain metabolism (FDG-PET) and blood flow changes (H215O-PET), but also the 3D determination of receptor distributions in fully conscious humans. Nearly every neurotransmitter system and neurochemical pathway can thereby be studied. The only limitations of ligand-PET studies are due to the complex chemical synthesis and therefore limited availability, often short half-life of the necessary radioactive compounds and
associated pressure of time during the experiments, the demanding hardware requirements and the frequent necessity of arterial cannulation. Altogether, ligand-PET studies are very time-consuming and usually only small amounts of volunteers/patients can be scanned.

In terms of ligand-PET in pain studies, particularly the opioidergic ligand tracers $[^{11}C]$-carfentanil ($\mu$-receptor specific agonist), $[^{11}C]$-diprenorphine (non-specific antagonist), $[^{18}F]$-diprenorphine (non-specific antagonist) und $[^{18}F]$-cyclofoxy ($\mu$- and $\kappa$-receptor antagonist) (Frost, 1993) are worth mentioning, but the NMDA-antagonistic tracers $[^{11}C]$-ketamine (Shiue et al., 1997) and $[^{18}F]$-memantine (Ametamey et al., 1999, 2002) are also of interest. Additionally, dopaminergic, serotonergic and muscarinic ligands, as well as PET-ligand tracers for studies of benzodiazepine and histamine receptors or the MAO-B system are available (Frost, 1993; Duncan, 1999). With this repertoire of tracers it is not only possible to display neuroanatomical receptor distributions, but ligand-PET has also the potential to evidence changes in receptor occupancy due to pharmacological/cognitive or other challenges and in pathological states such as chronic ongoing pain.

2. Exploring the opioid receptor system in the human brain

Opioid receptors were first demonstrated in the CNS by radioligand binding techniques in the rodent brain in the 1970s (Pert et al., 1976). Since then, in the pre-PET era, the characterization of the opioid receptor system was restricted to animal models or human post mortem tissue investigations. Even though these studies are still “gold-standard” with respect to receptor pharmacology, conclusions drawn with respect to function and human physiology were hampered by species differences, low numbers and/or the restriction to group comparisons.

Ligand-PET will revolutionize the exploration of neurochemical pathways in the way that the technique is performed in vivo, non-invasively and allows to investigate distinct challenges in the individual.

The early PET studies evidenced the anatomical brain distribution and local opioidergic receptor quantities in healthy subjects. Thereby, differences in the opioid receptor binding between the lateral versus medial pain system with higher binding in the medial pathway were confirmed (Jones, 1988, 1991). In addition to well known differences in receptor quantities depending on the brain regions (Hiller and Fan, 1996), ligand-PET also revealed considerable gender and age differences (Zubieta et al., 1999). $\mu$-opioidergic binding seems to increase with age in neocortical areas as well as in the putamen, and females show a higher opioidergic receptor density in a number of cortical and subcortical areas (anterior cingulate, prefrontal, parietal and temporal cortex, amygdala, thalamus, caudate, pons, cerebellum) (Zubieta et al., 1999). Furthermore, the basal expression of $\mu$-opioid receptors may depend on the genotype of the catechol-$\alpha$-methyl-transferase (COMT) val$^{108/158}$met polymorphism. The enzyme COMT is involved in the catalytic degradation of dopamine and noradrenaline and its gene on chromosome 22 codes two enzyme-isofoms: one soluble short (sCOMT) and a longer mem-brane-bound isoform (mbCOMT). A functionally relevant and common polymorphism leads to a substitution of valin (val) by methionin (met) at position 108 (sCOMT), respectively, 158 (mbCOMT) with reduction of the activity of the COMT enzyme. By means of ligand-PET, (Zubieta et al., 2003) were able to demonstrate concomitant increases in the amount of $\mu$-opioid receptors. Such effects of genotype on the receptor density were found in the anterior and pulvinar thalamus (and at lesser significance in the ventral basal ganglia) (Zubieta et al., 2003).

Thus, state-of-the-art opioid receptor ligand-PET now turn the tables: the basic characteristics of the opioid receptor system will more and more be studied in vivo and classical receptor autoradiography will merely have to confirm what is already known from functional imaging.

3. Exploring the opioidergic sites of action in the human brain

Beside the delineation of receptor distributions by ligand-PET techniques, water PET studies during infusion of $\mu$-opioidergic drugs (fentanyl and remifentanil) have shed light on the brain structures which are directly and indirectly modulated by synthetic opioids. Thereby, blood flow increases reflecting increased regional neuronal activity were detected among others in orbitofrontal, medial prefrontal and anterior cingulate cortex (ACC), particularly the perigenual portion of this brain region, as well as the midbrain (Firestone et al., 1996; Casey et al., 2000; Wagner et al., 2001). All these brain regions are well known to contribute to the processing of painful stimuli as well as attention and emotions. H$^{15}$O-PET experiments with simultaneous pain application during opioidergic infusion demonstrated a general suppression of pain induced activations by opioids (Casey et al., 2000). Concurrently, an activation of the anterior cingulate cortex was seen, which may indicate an active pain modulating role of this structure (Casey et al., 2000). Furthermore covariation of activity between the ACC and the PAG during pain and opioid analgesia, but not during pain alone has been shown (Petrovic et al., 2002). Placebo analgesia, which is known to be effective and to be reversible by naloxone administration, activated a similar neuronal network as opioids do, again including the ACC (Petrovic
et al., 2002). Thus, there is converging evidence for a network between ACC and brainstem structures mediating analgesia by synthetic opioids, but possibly also being involved in placebo and endogenous opioid analgesia.

Whereas $H_2^{15}$O-PET-studies or fMRI during pharmacological challenges are able to evidence cerebral activation sites of opioidergic drugs, by these means it cannot be answered to what extent increases of regional cerebral blood flow (rcBF) or blood oxygen-level dependent (BOLD) signal overlap with the receptor distribution in the brain. Combination of information derived from opioid receptor ligand-PET and activation PET/fMRI with exogenous agonists/antagonists will help to understand possible matches and mismatches of “action” (i.e., activation in $H_2^{15}$O-PET/fMRI) and receptor availability in these regions. First results again emphasize the role of the anterior cingulate cortex for the action of opioids (Sprenger et al., 2003).

4. Exploring opioid receptor function in clinical and experimental pain

In ligand-PET studies of clinical pain conditions, changes in the endogenous opioid system have been shown in a variety of brain structures. Thereby, studies during the pain state were either compared with sequential scans in the same individuals out of pain (Jones et al., 1994, 1999) or with a group of healthy volunteers (Willough et al., 2004). With relative consistency, decreases in ligand binding in association with present pain were reported in (pre)-frontal, insular, cingulated, parietal cortices and thalamus in trigeminal neuralgia, rheumatoid arthritis and central post stroke pain. Most of these structures are assumed to constitute the opioidergic matrix taking part in general pain processing and modulation. But indeed, the understanding of these signal changes between pain and non-pain conditions is limited thus far. Relative changes in binding potentials can be due to PET-ligand competition with endogenously released opioid peptides, opioid receptor internalization, regulation of receptor number or neuronal loss. These mechanisms might all as well as differentially contribute to these changes in distinct pain conditions. Furthermore, we cannot gather information about the modulation of downstream targets of opioid receptors by means of functional imaging techniques.

In the first published $[^{11}$C]-carfentanil study on experimental pain, sustained pain applied in the masseter muscles was used to evaluate short-term changes in the opioidergic system (Zubieta et al., 2001). In this study, reduced $\mu$-opioidergic binding due to an acute pain stimulus activation was reported in the dorsal anterior cingulate cortex, insula, thalamus, hypothalamus, amygdala and lateral prefrontal cortex. For the thalamus these results have been reproduced by another research group (Bencherif et al., 2002). Again, the decline in opioidergic binding induced by the experimental pain stimuli is mainly thought to be due to competition with endogenously released ligands and interpreted as “receptor activation”, but of course, receptor internalization may also play a role and the exact mechanism underlying these differences in binding are yet to be established (Laruelle, 2000).

Surprisingly, no “opioidergic activation” in the more rostral parts of the ACC have been reported by means of $[^{11}$C]-carfentanil-PET thus far. However, it needs to be mentioned that though the study paradigm used by Zubieta et al. was well designed, it is not possible to easily compare masseter pain with results from other imaging studies, mostly using phasic or tonic heat pain (Lorenz and Casey, 2002). It has to be awaited, whether further opioid displacement studies will confirm the lack of displacement, i.e., putative endogenous release in the rostral ACC or not. If activation of the rostral ACC could not or only partly be achieved by direct opioidergic neurotransmission, then activation would be imaginable via the amygdala, which showed marked displacement in Zubieta’s study, and which is reciprocally connected to the rostral ACC (Vogt and Pandya, 1987).

Zubieta also performed correlation analyses between sensory pain scores (McGill-Pain-Questionnaire) and activation of the opioidergic system and found negative correlations in the amygdala, the thalamus and nucleus accumbens. Similarly, activation of the thalamus, ACC and nucleus accumbens correlated negatively with affective pain ratings (Zubieta et al., 2001).

In addition to the above mentioned “static” neuroanatomical differences in opioidergic receptor density, dynamic ligand-PET further provided evidence that the $\mu$-opioid receptor mediated responses to pain stimuli also differ between genders (Zubieta et al., 2002). At identical pain magnitudes males exhibited greater opioidergic activation in the nucleus accumbens, the amygdala, the thalamus and the ventral pallidum/substantia innominata (Zubieta et al., 2002).

As a matter of fact, not only influences of opioids on cerebral activation patterns and the impact of pain on opioidergic neurotransmission and vice versa are today being investigated, but also interactions between neuroreceptor systems such as the dopaminergic and opioidergic system. Recently, it was shown that opioids directly modulate neurotransmission in the nigrostriatal dopaminergic pathway. Thereby, pharmacologically relevant doses of the $\mu$-agonist Alfentanil increased the binding potential of the dopamine D2 radioligand raclopride in the putamen and in the caudate nucleus (Hagelberg et al., 2002). This interaction between neuroreceptor systems is underlined by the mentioned influences of the COMT polymorphism on $\mu$-receptor densities and it is noteworthy that this polymorphism also leads to a
differential activation of the opioidergic system in striatopallidal circuits and other brain regions (cingulate cortex, thalamus, amygdala) in response to pain stimulation (Zubieta et al., 2003).

5. Conclusion and perspectives

PET studies using opioid ligands have shed light on the in vivo receptor distribution as well as dynamic changes effected by experimental and clinical pain conditions. Within the latter, the possibility to evidence stimulus-related neurotransmitter release is most fascinating. However, up to now there is a lack in the understanding of the basic mechanisms of “ligand activation”. Currently, it is assumed that short-term changes in PET-ligand binding might be related to competition of the endogenous transmitter with the radiolabelled ligand. However, this is only one of more possible assumptions. For example, changes might as well derive from ligand wash out due to stimulus-induced changes in regional blood flow. Further, agonistic PET-ligands itself may interfere with receptor desensitization or internalization and may rather hinder the detection of endogenous release.

Thus, there is a strong need for further systematic investigations. As one possible approach, these questions can be taken back to animal models, in which extent, time-course and localization of a putative endogenous transmitter release following acute stimulation can be demonstrated and characterized by complementary experimental approaches. Having at hand such a model of endogenous transmitter release, it will be possible to optimize PET-ligands in terms of affinity, specificity and intrinsic pharmacological activity and to modify PET modelling algorithms to depict such events in vivo.

A better understanding of the underlying mechanisms will then not only promote the interpretation of yet published results, but furthermore help to appropriately modify experimental strategies to maximize the signal changes in competition experiments and therefore, broaden the spectrum of potential applications.

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References


