PET imaging of brain sex steroid hormone receptors and the role of estrogen in depression
Khayum, Mohamed Abdul

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Chapter 7

Summary
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Estrogens and androgens are known to induce secondary sexual characteristics in females and males, respectively. However, these sex hormones can also exert profound effects on mood, mental state and memory. A decline in the circulating estrogens has been identified as a risk factor for premenstrual syndrome, post-natal depression, and post-menopausal depression. Likewise, the decrease in circulating androgens in middle-aged men (andropause) was also associated with a higher risk for brain disorders, like depression and Alzheimer’s disease. The effects of estrogens and androgens on brain function are processed by intricate mechanisms, affecting the synthesis and release of several other neurotransmitters. Estrogens and androgens mainly influence the serotonergic and dopaminergic system, but also the noradrenergic and cholinergic system are affected.

Estrogens and androgens act through their respective estrogen or androgen receptors. These receptors belong to the super-family of nuclear receptors. Sex hormone (nuclear) receptors migrate from the cytoplasm to the nucleus of the cell when they are activated by their respective ligands. In the nucleus of the cell, the activated receptors bind to specific binding sites in the promoter region of sex hormone responsive genes, resulting in activation or suppression of the transcription of these genes.

Estrogen and androgen receptors are widely distributed throughout the body. The sex steroid hormones can produce diverse biological effects, depending on the tissue in which the receptor is expressed. This thesis focuses on the central effects (effects on brain) of estrogens and androgens. Both estrogen and androgen receptors are expressed in the living mammalian brain. There are several techniques to measure the expression of these receptors, such as quantitative immunohistochemistry, immunohybridization, PCR and Western blot. However, these techniques cannot be applied on the living brain, as they are invasive in nature. Positron emission tomography (PET), on the other hand, can be employed to quantify the sex steroid hormone receptors in the living brain in a non-invasive manner. An introduction on the role of sex steroid hormones and their receptors in mental health and disease is provided in chapter 1 of this thesis.

In chapter 2, we discussed the available tracers for imaging of steroid hormone receptors by PET and Single Photon Emission Computed Tomography (SPECT). Almost all of these tracers have been developed and evaluated for detection of steroid hormone receptors in oncology. A few of these tracers for imaging of androgen and estrogen receptors have already entered into the clinical evaluation phase. So far, none of the available PET tracers have been validated for application as an imaging agent to monitor sex steroid hormone receptors in the brain. So we enumerated several PET and SPECT tracers that were developed for imaging of hormone sensitive tumors in this chapter and discussed the potential use of some of these tracers to detect the steroid hormone receptors in the brain. 16α-[18F]fluoro-17β-estradiol ([18F]FES), 21-[18F]fluoro-16alpha,17alpha-[(R)-(1′-alpha-furylmethylidene)dioxy]-19-norpregn-4-ene-3,20-dione ([18F]FFNP), and 16β-
[\textsuperscript{18}F]fluoro-5\alpha-dihydrotestosterone (\textsuperscript{18}F)FDHT) appear to be the most likely candidates for PET imaging of estrogen, progesterone and androgen receptors in the brain respectively.

The first part of this thesis describes the evaluation of the PET tracers 16\beta-[\textsuperscript{18}F]fluoro-5\alpha-dihydrotestosterone (\textsuperscript{18}F)FDHT) and 16\alpha-[\textsuperscript{18}F]fluoro-17\beta-estradiol ([\textsuperscript{18}F]FES) for in-vivo measurement of androgen and estrogen receptors in the brain respectively (\textit{chapters 3 and chapter 4}).

Androgens produce their central effect on mood and behavior through androgen receptors. However, it is not well known which areas of the brain express androgen receptors and how androgen receptor expression changes during disease. For that reason we evaluated \textsuperscript{18}F)FDHT as a tracer for monitoring the expression of androgen receptors in the brain, in orchiectomized and sham-orchiectomized male rats, using small animal PET imaging (\textit{chapter 3}). Ex-vivo biodistribution and PET studies showed that tracer uptake in the brain was low. No significant differences in tracer uptake between brain regions were observed, except for the pituitary that showed significantly higher uptake than other regions. \textsuperscript{18}F)FDHT uptake in the surrounding cranial bones was high and increased over time, likely due to extensive defluorination of the tracer. The uptake of radioactivity in the cranial bones caused significant spill-over effects into the brain and consequently peripheral brain regions had to be excluded from the PET data analysis. Metabolism of \textsuperscript{18}F)FDHT was very fast. The tracer was more rapidly metabolized in orchiectomized than in sham-orchiectomized rats. Results of pharmacokinetic analysis, revealed \textsuperscript{18}F)FDHT follows two compartment reversible kinetics in the brain. The brain uptake of \textsuperscript{18}F)FDHT could not be blocked by endogenous androgens or exogenous dihydrotosterone. The conclusion of this study was therefore that it was not feasible to image brain androgen receptors with \textsuperscript{18}F)FDHT PET in rats, because of the lack of specific binding, low brain accumulation and fast degradation of the tracer.

Estrogens affect brain functions through estrogen receptors in the brain. Currently, no method is available to investigate the expression of estrogen receptors in the brain in a non-invasive fashion. \textsuperscript{18}F)FES PET may be a suitable tool to measure the expression of estrogen receptors in the living brain. In \textit{chapter 4}, female rats in the proestrus and diestrous phase, ovariectomized female rats, ovariectomized female rats co-injected with 17\beta-estradiol and male rats were used to evaluate the feasibility of \textsuperscript{18}F)FES PET to monitor ER expression in the brain and how this is affected by different levels of circulating estrogens. The pituitary showed the highest \textsuperscript{18}F)FES uptake, followed by the hypothalamus, bed nucleus of the stria terminalis and the amygdala. \textsuperscript{18}F)FES uptake was significantly higher in pituitary and hypothalamus of ovariectomized rats then in untreated rats in the proestrous phase. Administration of 17\beta-estradiol to ovariectomized rats resulted in a reduction in \textsuperscript{18}F)FES uptake in pituitary and hypothalamus. Pharmacokinetic modeling showed higher total volume of distribution ($V_T$) and non-displaceable binding potential ($BP_{ND}$) in pituitary than in other brain regions for all groups. $V_T$ and $BP_{ND}$
were not statistically significant between groups for any brain region. The conclusion of this study was that $[^{18}\text{F}]$FES PET could only be used for imaging of estrogen receptors in brain areas with high levels of estrogen receptor expression.

Since imaging of subtle differences in sex steroid hormone receptors with PET in rats appeared to be highly challenging, the second part of this thesis focused on studying the effect of estrogen levels on behavior and brain metabolism in ovariectomized rats, as a model for menopause (chapter 5 and chapter 6).

Menopause is associated with a higher incidence of mental disorders, decline in cognition, anxiety, depression and certain neurodegenerative disorders like Alzheimer’s and Parkinson’s disease. Replacement of estrogens in post-menopausal women may result in an improvement in cognitive abilities and a decrease in signs of depression. However, the time at which estrogen replacement is initiated appears to be a critical factor. Thus, in chapter 5 the effect of immediate and 1 week delayed estradiol replacement on depressive-like behavior in ovariectomized rats was studied using the forced swim test (FST). In addition, the effect of estradiol replacement on brain glucose metabolism was studied in resting state and under an unavoidable stress condition, i.e. during the forced swim test. Measurement of brain glucose metabolism was performed by $[^{18}\text{F}]$FDG PET. The effects of estradiol replacement on depressive-like behavior and the brain glucose metabolism were compared with the effects of sub-acute treatment with the standard antidepressant drugs fluoxetine and escitalopram. Estradiol replacement immediately after ovariectomy, but not after a 1-week delay, could reduce depressive-like behavior in the forced swim test and reduced whole brain glucose metabolism in resting state, but not during FST induced stress. The whole brain uptake of $[^{18}\text{F}]$FDG in ovariectomized rats was higher in the resting state than during the FST (stress), irrespective of whether they received estrogen replacement or not. No effect of estrogen replacement on whole brain uptake of $[^{18}\text{F}]$FDG was found either at resting state or during FST. The focal changes in the brain glucose metabolism as measured by voxel based analysis showed significant changes in relative brain glucose metabolism between resting state and during FST. Application of FST stress in general was found to affect the brain areas associated with emotion, coordination of muscular movement, maintenance of balance and posture to any (stressful) condition in comparison to resting state. Between group comparison of $[^{18}\text{F}]$FDG PET acquired after FST-induced stress showed significant focal differences in brain glucose metabolism between rats that received immediate estradiol replacement and placebo treated ovariectomized rats. In particular, ovariectomized rats receiving immediate estradiol replacement showed an increase in brain glucose metabolism in fear processing areas, like periaqueductal grey, superior colliculus and cerebellum, as compared to placebo treated ovariectomized rats. Therefore we concluded that estradiol replacement immediately after ovariectomy could produce a significant antidepressant effect, which was accompanied by increase in the glucose metabolism in fear processing areas of the brain during FST stress. Remarkably, both standard antidepressant drugs did not show any significant effect on depressive-like behavior.
compared to placebo treated rats and only escitalopram treated animals could affect brain metabolism.

Not all post-menopausal women suffer from depression and therefore the cause of post-menopausal depression is likely multifactorial. Stress could be a potential contributor to the induction of depressive-like symptoms in post-menopausal women. So, in chapter 6, we studied the effect of chronic estrogen depletion and chronic mild stress (CMS) on depressive-like behavior, anxiety and brain glucose metabolism in ovariectomized female rats. Estrogen depletion by ovariectomy for two weeks did not induce any significant signs of depressive-like behavior nor anxiety, but signs of depressive-like behavior were evident 8 weeks after ovariectomy. Exposure to CMS for 6 weeks did not aggravate the signs of depressive-like behavior in ovariectomized rats. Estrogen depletion had no effect on global brain metabolism, neither 2 weeks nor 8 weeks after ovariectomy. However, both 2 weeks and 8 weeks of estrogen depletion induced focal changes in brain glucose metabolism as was assessed by voxel based analysis of the $[^{18}\text{F}]$FDG PET images. At both time points a decrease in glucose metabolism in the basal ganglia, limbic system and regions related to cognition and emotion was observed in estrogen depleted rats, as compared to rats that received estrogen replacement. Estrogen depleted rats showed an increase in glucose metabolism in cortical regions. CMS had no effect on brain glucose metabolism, neither in estrogen depleted rats nor in rats that received estradiol replacement. Thus, we concluded that ovariectomy could induce depressive-like behavior and changes in brain glucose metabolism, especially in brain areas associated with cognition and emotion, but CMS could not.

In conclusion, in the first part of the thesis we found that, with $[^{18}\text{F}]$FES, we were able to image the estrogen receptor rich areas of the brain, but that $[^{18}\text{F}]$FDHT was not a suitable PET tracer for imaging of brain androgen receptors. In the second part of the thesis we found that estrogen replacement initiated immediately after ovariectomy was beneficial in reversing depressive-like behavior in rats and affected brain metabolism in ovariectomized rats. The regional pattern of brain glucose metabolism suggests that immediate estradiol replacement influences motivational cues. Additionally, chronic estrogen depletion induced depressive-like behavior that increased over time and affected glucose metabolism in brain areas involved in emotion. However, chronic mild stress did not add to depressive-like behavior or brain glucose metabolism.