Fundamental studies on radiotracers intended for receptor imaging in dementia

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CHAPTER 7

THE EFFECTS OF REPEATED ADMINISTRATION OF NEUROLEPTICS ON MUSCARINIC RECEPTOR BINDING IN RAT BRAIN, AS MEASURED BY $[^{123}I]$IODODEXETIMIDE

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Abstract

In vivo muscarinic receptor imaging, such as \([^{123}I]\text{Iododexetimide SPECT}, may be of value to select cognitively compromised patients that could benefit from acetylcholinesterase inhibitors, for instance patients suffering from dementia or schizophrenia. Atypical neuroleptics are frequently used by these patients and may influence the binding of muscarinic receptor tracers in the brain. In this study, we examined the effects of repeated administration of neuroleptics on the biodistribution of \([^{123}I]\text{Iododexetimide in the rat brain.}

The atypical neuroleptics olanzapine (n=8 rats; 2.5mg/kg body weight) or risperidone (n=9; 2.5 mg/kg) were administered orally once daily, during 14 consecutive days before injection of \([^{123}I]\text{Iododexetimide}. Tracer uptake in muscarinic receptor-rich brain areas (i.e. cortical areas and striatum) and in brain areas expressing relatively low levels of muscarinic receptors (i.e. hypothalamus) was compared to data obtained from control rats (n=13). The tracer was injected 24h after the last administration of the drugs to exclude direct competitive effects on tracer binding.

A significant increase in tracer binding, expressed as a ratio to cerebellar binding, was found only in the hypothalamus after risperidone treatment as compared to the control group (P=0.004). Repeated administration of olanzapine did not result in any changes in central muscarinic receptor availability.

Since in-vivo studies in humans can only accurately assess muscarinic binding in muscarinic-rich brains areas, our data suggest no large non-acute effects of olanzapine and risperidone on muscarinic receptor imaging in humans using the non-subtype selective radiotracer \([^{123}I]\text{Iododexetimide.}

Introduction

Imbalance of the cholinergic neurotransmitter system is present in several neurologic and neuropsychiatric diseases. In the central nervous system, this neurotransmitter system plays a key role in processes such as cognitive processing, memory and attention. Particularly the muscarinic component of the cholinergic system is known to be affected in various forms of dementia. In Alzheimer’s Disease (AD), Dementia of the Lewy Body type (DLB) and Parkinson’s Disease related Dementia (PDD), a marked decrease of muscarinic receptor density has been shown. Moreover, growing evidence suggests dysfunction of the muscarinic
receptor system in schizophrenia$^3$. Most current FDA-approved pharmacological treatments for dementia are based on the enhancement of the cholinergic neurotransmitter acetylcholine (ACh) in the synaptic cleft in order to improve cognitive function. These treatments are also tested in schizophrenic patients who show cognitive decline. Improvement of cognition is pursued by inhibition of acetylcholinesterase, which normally breaks down ACh. In patients with dementia, modest improvement of cognition by acetylcholinesterase inhibitors (AChEIs) has been demonstrated whereas the effects on the cognition of schizophrenic patients are modest at best$^3$. Besides AChEIs, cholinergic agonists may also improve cognition of schizophrenic patients, particularly muscarinic M$_1$ receptor subtype selective agonists, which may also have direct antipsychotic effects$^3$. Efficacy of cholinergic medication may be improved by better selection of patients who could benefit from these drugs. This selection process may be facilitated by imaging studies by means of PET or SPECT using radiotracers for muscarinic receptors (M$_1$-5). Although many of these tracers have been evaluated in the past, none has proven to be fully selective or otherwise suitable for imaging of single muscarinic receptor subtypes in the living human brain to date. However, regional changes of total muscarinic receptor density in the brain of demented or schizophrenic patients have been shown using non- or partly selective muscarinic receptor tracers, including iododexetimide$^{4,5}$. \[123I\]Iododexetimide ((S)-(+)\-3-phenyl-3-(4-piperidinyl)-2,6-piperidinedione((S)-nordexetimide) was previously developed and evaluated as a non-subtype selective muscarinic receptor SPECT-tracer for imaging of muscarinic receptors in the living human brain$^{6,7}$. Potential effects of psychoactive drugs on the binding of this, or future subtype selective muscarinic receptor tracers should be investigated since patients or study subjects may already be medicated when a brain muscarinic SPECT or PET study is performed.

Atypical or second generation neuroleptics, such as olanzapine and risperidone, are the therapy of choice for schizophrenic patients but are also prescribed frequently to demented patients to control psychotic symptoms, although serious side-effects may occur particularly in DLB patients. These types of neuroleptics have a complex pharmacologic profile and while these drugs primarily target the serotonergic and dopaminergic neurotransmitter system, actions on the muscarinic system have been demonstrated. Earlier studies have indeed suggested a
competitive effect of concurrently used neuroleptics on $^{123}$Iiododexetimide and $^{123}$I-QNB SPECT studies. Chronic administration of these types of medication, however, may also influence the binding of muscarinic receptor tracers in the brain due to changes in muscarinic receptor density as a result of up- or downregulation. This may also complicate the interpretation of imaging studies on muscarinic receptor densities, even if the medication is discontinued shortly before the brain scan.

In this study, we therefore examined the effects of repeated administration, during 14 consecutive days, of the atypical neuroleptics olanzapine and risperidone on $^{123}$Iododexetimide binding in the rat brain. The radiotracer was injected 24h after the last administration of the drugs to exclude direct competitive effects on tracer binding.

**Materials and methods**

To study the effects of repeated administration of the neuroleptics risperidone and on the distribution of $^{123}$Iododexetimide in the rat brain, male Wistar rats (270-325g, obtained from Harlan, Horst, The Netherlands) were used (8-13 per group). Pre-medication was administered to awake rats during 14 consecutive days, but not on the day of the dissection experiment to prevent direct competitive effects of the administered medication itself or acutely increased ACh levels in the brain induced by the medication.

The medication was given orally once daily in 1mL saline. All medication has been shown to be centrally active after oral administration to rats in the used dosages. Groups of rats received placebo (saline only, n=13), risperidone (2.5mg/kg bodyweight, n=9) or olanzapine (2.5mg/kg bodyweight, n=8). No serious side-effects were observed after treatment of the animals.

Injection of the radiotracer and the dissection experiment was performed on the 15th day of the experiment, to exclude direct competitive effects of medication on tracer binding. $^{123}$Iododexetimide (specific activity 185 MBq/nmol; radiochemical purity 98.0%) was a generous gift from GE Healthcare, Eindhoven, The Netherlands. Its radiosynthesis was described earlier. On the day of the dissection experiment, all rats received approximately 3.7MBq $^{123}$Iododexetimide in 0.3mL phosphate buffer with 7.8% ethanol (pH 5.9) intravenously via a lateral tail vein. All rats were sacrificed at 2h after injection of the radiotracer by bleeding via heart puncture. On
this day, the procedures were performed under ketamine/ xylazine (2:1, i.m.) anesthesia. Blood, fat, muscle, organs and various brain structures (frontal cortex, occipital cortex, striatum, hippocampus, amygdala, thalamus, hypothalamus, pons) were rapidly excised and weighted separately. The $^{123}$I radioactivity of each structure was assayed in a gamma counter (Minaxi $\gamma$ A5530, Canberra-Packard). Data were corrected for radioactive decay, brain structure weight for each rat and were eventually expressed as the percentage of the injected dose, multiplied by the body weight in grams, per gram tissue or blood (%ID* g/g)\(^{11}\). In the brain, the cerebellum was chosen as the reference area, since it expresses a relatively low density of muscarinic receptors\(^{12-14}\). Tracer uptake in brain structures was expressed as the ratio to the cerebellar uptake ([brain structure]/[cerebellum]) for each rat.

The performed experiment is in agreement with The Dutch Experiments on Animals Act (1977) and was approved by the Animal Ethics Committee (AMC, Amsterdam, The Netherlands).

**Statistical analysis**

Differences in radioactive uptake of organs and brain structures between groups, as well as differences in binding ratios of multiple brain structures to the cerebellum between groups were analyzed using SPSS 15.0 by analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons. Probability values <0.01 were considered significant.

**Results**

In all studied groups, a high uptake of radioactivity was measured in the liver and the lungs at 2h after injection. Kidneys, fat tissue, spleen and heart showed moderate radioactive concentrations. Muscle and blood samples contained low concentrations of radioactivity.
In contrast to the risperidone group, a significantly higher radioactive uptake in liver \((P=0.001)\) and kidney \((P=0.005)\) was detected in the olanzapine group. Also in fat tissue, a significantly higher uptake was detected after treatment with olanzapine. Although not statistically significant, the pre-treated groups showed slightly higher absolute radioactive concentrations in all other peripheral structures, as compared to the control group (Figure 7.1.).

In the brain, all study groups showed a high absolute uptake of radioactivity in the cerebral (frontal and occipital) cortex, striatum, hippocampus and amygdala. Thalamus, hypothalamus and pons displayed a lower tracer accumulation. As expected, the lowest radioactive concentrations were measured in the cerebellum (Figure 7.2.). A significant increase in absolute radioactive uptake after repeated treatment with risperidone or olanzapine was revealed only in the muscarinic receptor deprived hypothalamus \((P=0.001)\) of the risperidone treated animals as compared to the control group. The calculated brain structure-to-cerebellum ratios also showed a statistically significant higher hypothalamus-to-cerebellum ratio in the risperidone treated group as compared to the control group.

**Figure 7.2.** Absolute uptake of radioactivity in brain structures in control animals \((n=13)\), olanzapine treated animals \((n=8)\) and risperidone \((n=9)\) treated animals. Data are expressed as the percentage of the injected dose, multiplied by the body weight in grams, per gram tissue or blood \((%ID^*g/g)\). Data are expressed as the average specific binding ratio \(\pm 1\) standard error of the mean (SEM).

* \(P=0.001\) (increase in uptake as compared to control group)

* ** \(P=0.004\) (increase in ratio as compared to control group)

Discussion

In this study, we examined the effects of repeated administration of the atypical neuroleptics olanzapine and risperidone on the in-vivo binding of \([^{123}\text{I}]\)Iododextemide. The biodistribution of \([^{123}\text{I}]\)Iododextemide in the rat brain was consistent with the distribution of muscarinic receptors \(^{12-14}\). Compared to controls, repeated administration during 14 days of the tested drugs did not induce major changes in pattern of radiotracer...
er distribution in the rat brain at 24h after the last administration. Surprisingly however, a statistically significant increase in tracer uptake ratio to the cerebellar uptake was detected only in the hypothalamus of risperidone treated rats.

The pharmacologic mechanisms of atypical neuroleptics such as risperidone and olanzapine, are complex and multiple neurotransmitter systems seem to be involved. Unlike typical neuroleptics such as haloperidol, the primary target receptor of atypical neuroleptics is not the dopamine D2 receptor, but the serotonin 5-HT2A receptor, although D2-antagonism is also a property of all atypical neuroleptics. Olanzapine also binds with high affinity to muscarinic receptors where it acts predominantly but not exclusively as a muscarinic antagonist. Risperidone has a very low affinity for muscarinic receptors. Indeed, previous studies showed higher in-vivo occupancy of muscarinic receptors by olanzapine than risperidone in schizophrenic patients on medication. After repeated or chronic administration of olanzapine, an upregulation of several central muscarinic receptor subtypes would be expected to occur because of the antagonistic properties of the neuroleptic. While not shown by the present results, Terry and co-workers recently reported a modest increase of [3H]AFDX-384 binding, suggesting an increase in muscarinic M2 receptor availability, in many rat brain structures including the hippocampus after 90 but not 180 days of chronic oral treatment with olanzapine. No alteration of muscarinic M1/M4 receptors was detected with [3H]pirenzepine and after a drug washout period of 2 weeks. Interestingly, in the present study repeated administration of olanzapine also did not induce significantly higher cortex-to-cerebellum or hippocampus-to-cerebellum ratios of [123I]Iododexetimide binding in the rat brain. Importantly, also absolute binding in the cerebellum, representing non-specific binding, was not influenced by olanzapine. However, one has to take into account the non-selective binding profile of [123I]Iododexetimide for all muscarinic receptor subtypes. Since the densities of the M2-5 receptor subtypes are less abundant in the (rat) brain than the M1 subtype, in-vivo [123I]Iododexetimide mainly represents binding to M1 receptors. Therefore, our present results are in agreement with the abovementioned results obtained with [3H]pirenzepine, suggesting that upregulation of muscarinic M1 receptors in the brain had not occurred after repeated administration of olanzapine. Animal studies suggest a reversible and subtype-specific upregulation of muscarinic receptor availability after prolonged treatment with olanzapine. While the brain half-life of olanzapine in rats is not known, the
results of the present study suggest that, if at all, only small concentrations of olanzapine or relevant metabolites are present after 24h. We cannot exclude, however, that an upregulation in muscarinic receptor availability is counterbalanced at the time of study (>24h after last dosing) by the residual effects of the muscarinic antagonist olanzapine. Additionally, a potential limitation of our study design is that we are able to calculate only absolute uptake values, therefore we cannot exclude that an increase in $B_{\text{max}}$ is counterbalanced by an increase in $K_d$.

In a previous study we showed, as expected, that acute administration of scopolamine was able to block $[^{123}\text{I}]$Iododexetimide binding to muscarinic receptors in rat brain\textsuperscript{19}. However, we additionally performed a pilot study in which we orally administered 1mg/kg scopolamine, a potent non-selective muscarinic receptor antagonist, repeatedly during 14 days to four rats. Using exactly the same protocol as the present study (including a 24h washout period to exclude direct competitive effects on radiotracer binding), no statistically significant differences were detected as compared to the control group in any of the brain structures or brain structure-to-cerebellum ratios (unpublished results). Again, this is in line with the abovementioned report by Terry et al. However, in the pilot experiment a statistically significant increase of $[^{123}\text{I}]$Iododexetimide binding was detected in the heart as compared to the control group, which may reflect peripheral upregulation of total muscarinic receptors whereas in the present study, a significant upregulation could not be shown in the rat heart after repeated administration of olanzapine.

Risperidone has a very low affinity for muscarinic receptors, and therefore upregulation of muscarinic receptors due to chronic antagonism would not be expected to occur. Interestingly, in the present study a significantly higher absolute tracer uptake in the hypothalamus, and as a consequence a significantly higher hypothalamus-to-cerebellum ratio, in risperidone treated animals was observed. In the other examined brain structures that are known to express similar densities of muscarinic receptors (i.e. amygdala and thalamus), a slight increase in brain structure-to-cerebellum ratio was revealed. In brain structures that express a high density of muscarinic receptors, such differences in the ratios to the cerebellar uptake were not shown. Although regional differences in pharmacological actions of risperidone cannot be excluded, the reason for this observation in the hypothalamus remains unclear.

Since the absolute uptake in the brains of risperidone treated rats was slightly higher in muscarinic receptor-rich areas, as compared to both the control and the olanzapine treated group, this suggests an increase

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in specific binding. One possible other explanation for these observations in the risperidone treated rats may be an enhancement of tracer delivery due to an increase in brain perfusion, although such mechanism has not been described. Another possibility may be an impairing effect of risperidone to brain metabolism of drugs (such as $^{[123]}$Iododextremide), which may prolong the presence of unmetabolized tracer in the brain in this study, although evidence from the literature that support this hypothesis is lacking.

In rodents, acute administration of both olanzapine and risperidone induces an increase of ACh-levels in many brain areas\textsuperscript{20,21}. Olanzapine has been shown to induce a larger increase of ACh concentrations in the brain than risperidone, although such enhancement of ACh efflux was not detected in the striatum\textsuperscript{20}. An increase of the ACh concentration in the synaptic cleft, which would be expected due to the antagonistic properties of olanzapine, may prevent upregulation of muscarinic receptors to occur and this may explain why $^{[123]}$Iododextremide binding did not show an important enhancement in the brain after treatment with olanzapine. Given that olanzapine increases ACh efflux in the synaptic cleft much more potently than risperidone\textsuperscript{21}, this prevention of an upregulation probably has a larger impact on the data obtained from olanzapine treated animals. An increase of ACh in peripheral structures due to olanzapine or risperidone is unlikely, and the small increases in $^{[123]}$Iododextremide uptake in peripheral organs (such as the heart) may therefore be the result of upregulation of muscarinic receptors.

Peripherally, high concentrations of the radiotracer were detected in the excretory organs (liver>kidneys), confirming the lipophilicity and consequently primarily hepatobiliary excretion route of $^{[123]}$Iododextremide and its metabolites. A slightly higher radiotracer accumulation was detected in these organs in the drug treated groups (significantly higher in the olanzapine group), which may reflect impairing effects of the repeatedly administered drugs to hepatic drug clearance which may decelerate the excretion of the tracer or its metabolites. In this study we did not measure plasma metabolites to support this hypothesis, but the higher absolute levels in the organs may be also explained by such mechanism. In particular, the significantly higher uptake in fat tissue after repeated treatment with olanzapine suggests prolonged peripheral presence of $^{[123]}$Iododextremide (or lipophilic metabolites) of the tracer in rats. Impaired hepatic metabolism of the tracer may therefore also partly explain the apparent, slightly higher absolute tracer uptake in rat brain structures of treated rats. Although severe hepatotoxic effects of olan-
Zapine are rare, an asymptomatic increase in liver enzymes has been shown in humans for both olanzapine and risperidone. Also in rats, a previous study by Patel and co-workers reported a significant increase of serum glutamine pyruvate transaminase (SGPT or ALT) after treatment with high oral doses (20mg/kg daily) of olanzapine, which may reflect damage to hepatocytes, although the authors classified the drug as relatively non-toxic. In the present study, the administered olanzapine doses were substantially lower, which may render hepatotoxicity by this drug less likely. For risperidone, direct hepatic damage was not shown by Halici and co-workers at cellular level after chronic administration of the drug during 6 weeks (daily, 1mg/kg intraperitoneally). All drugs that were used in the present study have been shown to be centrally active in rats in earlier reports, using drug doses in the same range. Drug doses were well above the normal human doses. However, we did not attempt to simulate a human dosing scheme in this study, since the short half-lives of the medication in rodents ideally requires continuous infusion or multiple daily administrations of the drugs. Metabolism of risperidone is faster than the metabolism of olanzapine in rats (in plasma 1h and 2.5h, respectively). However, a difference in dissociation rate between the half-life of the drugs in the brain and in plasma has been shown previously. In the study by Tausher et al., half-life times in the human brain of olanzapine and risperidone was approximately 3 and 6 times longer than in plasma. Although it's uncertain whether these data can be extrapolated to rodents, this phenomenon could contribute to the occurrence of upregulation of muscarinic receptors in olanzapine treated rats. Alternatively, a prolonged presence of olanzapine and risperidone in the brain will also induce prolonged ACh-release due to these drugs, which may prevent upregulation of muscarinic receptors to occur. Moreover, if a slower dissociation rate of neuroleptics is present in the rat brain, small direct competitive effects of unmetabolized olanzapine may have occurred in the present study. Since it was not the scope of the present study, we did not perform brain or plasma-metabolites measurements. Nor did we perform a Scatchard-plot to determine the Bmax for the same reasons. Further studies should be performed to elucidate the exact pharmacology of olanzapine and risperidone in the rat brain. Finally, effects of the administered anesthesia on the binding of [125I]iododextemide may have influenced the present study. However, the pattern of iododextemide binding matched the distribution of muscarinic receptors in the rat brain. Moreover, we have reported earlier that...
ketamine/xylazine anesthesia does not influence the binding pattern of a potential muscarinic M2-receptor tracer as compared to hypnorm/midazolam. If effects of the anesthesia on the binding of the radiotracer are present, these effects are considered systematic, since the same anesthesia protocol was used for all rats.

Human studies have shown the feasibility of non-selective muscarinic receptor tracers to detect changes in total muscarinic receptor densities in patients suffering from schizophrenia or dementia in-vivo. Although direct competitive effects of olanzapine on [123I]Iododextimide binding in the brain of schizophrenic patients may influence muscarinic receptor SPECT imaging, the effects of chronic exposure to these drugs on [123I]Iododextimide binding in the human brain are yet unknown. From the present study we conclude that in rats, repeated administration of olanzapine or risperidone does not substantially influence the muscarinic receptor availability as measured by [123I]Iododextimide in brain areas expressing high densities of muscarinic receptors.

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