[123]I-Iododexetimide Preferentially Binds to the Muscarinic Receptor Subtype M$_1$ In Vivo

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The muscarinic M$_1$ receptor (M$_1$R) is highly involved in cognition, and selective M$_1$ agonists have procognitive properties. Loss of M$_1$R has been found in postmortem brain tissue for several neuropsychiatric disorders and may be related to symptoms of cognitive dysfunction. ^{123}I-iododexetimide is used for imaging muscarinic acetylcholine receptors (mAchRs). Considering its high brain uptake and intense binding in M$_1$R-rich brain areas, ^{123}I-iododexetimide may be an attractive radiopharmaceutical to image M$_1$R. To date, the binding affinity and selectivity of ^{123}I-iododexetimide for the mAchR subtypes has not been characterized, nor has its brain distribution been studied intensively. Therefore, this study aimed to address these topics. **Methods:** The in vitro affinity and selectivity of [127]I-iododexetimide (cold-labeled iododexetimide), as well as its functional antagonist properties (guanosine 5′-[γ-^{35}S-thio]triphosphate [GTPγ[35]S] assay), were assessed on recombinant human M$_1$R–M$_5$R. Distributions of [127]I-iododexetimide and ^{123}I-iododexetimide in the brain were evaluated using liquid chromatography–mass spectrometry and storage phosphor imaging, respectively, ex vivo in rats, wild-type mice, and M$_1$–M$_5$ knock-out (KO) mice. Inhibition of [127]I-iododexetimide and ^{123}I-iododexetimide binding in M$_1$R-rich brain areas by the M$_1$R/M$_4$R agonist xanomeline, or the antipsychotics olanzapine (M$_1$R antagonist) and haloperidol (low M$_1$R affinity), was assessed in rats ex vivo. **Results:** In vitro, [127]I-iododexetimide displayed high affinity for M$_1$R (pM range), with modest selectivity over other mAchRs. In biodistribution studies on rats, ex vivo [127]I-iododexetimide binding was much higher in M$_1$R-rich brain areas, such as the cortex and striatum, than in cerebellum (devoid of M$_1$Rs). In M$_1$ KO mice, but not M$_2$–M$_5$ KO mice, [127]I-iododexetimide binding was strongly reduced in the frontal cortex compared with wild-type mice. Finally, acute administration of both an M$_1$R/M$_4$R agonist xanomeline and the M$_1$R antagonist olanzapine was able to inhibit ^{123}I-iododexetimide ex vivo, and ^{123}I-iododexetimide binding in M$_1$-rich brain areas in rats, whereas administration of haloperidol had no effect. **Conclusion:** The current results suggest that ^{123}I-iododexetimide preferentially binds to M$_1$R in vivo and can be displaced by M$_1$R ligands. ^{123}I-iododexetimide may therefore be a useful imaging tool as a way to further evaluate M$_1$R changes in neuropsychiatric disorders, as a potential stratifying biomarker, or as a clinical target engagement biomarker to assess M$_1$R.

Cholinergic neurotransmission plays an important role in maintaining cognitive function, as shown by the profound cognitive deficits caused by administration of muscarinic acetylcholine receptor (mAchR) antagonists (1) and the broad clinical use of cholinesterase inhibitors to maintain or improve cognitive function in mild to moderate Alzheimer disease, a disease characterized by loss of cholinergic neurons (2). Abnormalities in the cholinergic system have also been hypothesized to contribute to cognitive dysfunction in schizophrenia, as, for example, deficits in mAchR expression have been observed in postmortem tissue from a subset of schizophrenic patients (3), whereas xanomeline, a muscarinic agonist, was shown to improve cognitive function in a pilot study on patients with schizophrenia (4–6).

Of the 5 human mAchR subtypes (M$_1$–M$_5$), the M$_1$ receptor (M$_1$R) is most abundant in the brain (7–10), displaying the highest expression in regions relevant to cognition such as the hippocampus and cerebral cortex, as well as subcortical areas such as the striatum (11–13). Pharmacologic imaging studies, electrophysiologic studies, and behavioral animal studies further support a role for M$_1$Rs in learning and memory (14–16). In this regard, recently developed drugs selectively targeting M$_1$R, including M$_1$ agonists and positive allosteric modulators, have helped to further define the role of this receptor subtype in cognition (5,8–10), providing further impetus for clinical development of therapies for diseases associated with cognitive deficits.

Thus, to probe changes in M$_1$R expression in disease and potential patient stratification or target engagement biomarkers, imaging of M$_1$R may be of considerable value. ^{123}I-iododexetimide has been used to image mAchR in vivo with SPECT (17,18). However, this radiotracer has not been well characterized pharmacologically, and only a few studies have described its distribution in the rodent brain. Taking into account the high expression of M$_1$R in the brain (7), the high binding of ^{123}I-iododexetimide in brain areas expressing M$_1$R (19,20), and its high brain uptake (7%–8%) (19), we were interested in establishing whether the in vivo binding of this

tracer predominantly reflects binding to the M$_1$R subtype. Therefore, we have characterized ^{123}I-iododexetimide binding to mAchRs by studying, first, the in vitro binding selectivity across recombinant human M$_1$R–M$_5$R; second, ex vivo binding in rats, wild-type mice, and M$_1$R–M$_5$R knockout (KO) mice using liquid chromatography with mass spectroscopy (LC-MS/MS) and storage phosphor imaging; and last, the effects of an M$_1$ agonist and antipsychotics with high and low affinity for M$_1$R (olanzapine and haloperidol, respectively) on ex vivo ^{123}I-iododexetimide binding in rats. We anticipate that our data will support the hypothesis that in vivo binding of $^{123/127}$I-iododexetimide predominantly reflects binding to M$_1$R.

MATERIALS AND METHODS

^{127}I-iododexetimide was prepared by Eli Lilly and Co. For the ^{127}I-iododexetimide experiments, male Sprague–Dawley rats were purchased from Harlan (weight range, 200–300 g). Male C57Bl6J mice (15–34 g) and muscarinic KO animals for M$_1$R (line 1784), M$_2$R (line 1454), M$_3$R (line 1455), M$_4$R (line 1456), and M$_5$R (line 1457) (15–47 g) were purchased from Taconic. For the ^{123}I-iododexetimide studies, male Wistar rats (200–250 g) were obtained from Harlan. ^{3}H-n-methylscopolamine (specific activity, 3.16 TBq/mmol) and Chinese hamster ovary cell membranes expressing human M$_1$–M$_5$ mAChR were obtained from Perkin Elmer.

All animals were group-housed and provided with food and water ad libitum. A normal 12 h/12 h light/dark cycle was applied with lights on at 7:00 AM.

In Vitro Competitive Binding Experiments

To investigate binding selectivity, the affinity of ^{127}I-iododexetimide was examined across mAchR subtypes M$_1$–M$_5$. Chinese hamster ovary cell membranes overexpressing human mAchR M$_1$–M$_5$ subtypes were incubated with ^{3}H-n-methylscopolamine in the presence or absence of 11 different concentrations of ^{127}I-iododexetimide. The concentration–effect data were curve-fit to derive the potency (inhibitory concentration of 50% [IC$_{50}$]) of ^{127}I-iododexetimide. The inhibition constant (K$_i$) of ^{127}I-iododexetimide was then calculated by the Cheng–Prusoff equation: K$_i$ = IC$_{50}$/(1 + ([L]/K$_d$)), where L is concentration of free radioligand (21). Further details are provided in the supplemental materials (available at http://jnm.snmjournals.org).

To assess functional antagonism of ^{127}I-iododexetimide, further in vitro profiling was performed using a GTPγ^{35}S binding methodology. Membranes acquired from Chinese hamster ovary cells overexpressing human mAchR subtypes were used at 20 mg/well. An 80% effective acetylcholine concentration (20 μM) was used to assess antagonism. Further details are provided in the supplemental materials.

LC-MS/MS Biodistribution Studies

To examine the brain distribution of ^{127}I-iododexetimide, a 10 μg/kg bolus of ^{127}I-iododexetimide was injected into the lateral tail vein of awake rats (n = 4 per group), who were sacrificed by cervical dislocation after 5, 10, 20, and 60 min. The frontal cortex, striatum, cerebellum, and plasma were collected from each animal. To evaluate specific binding in vivo, groups of rats (n = 3–4) received a dose of the M$_1$R/M$_4$R agonist xanomeline (1–60 mg/kg subcutaneously) or vehicle. Thirty minutes later, the animals received a 10 μg/kg bolus of intravenous ^{127}I-iododexetimide. Forty minutes afterward, the animals were sacrificed and the same tissues as for the brain distribution study were collected.

To further investigate the composition of specific binding to mAchR subtypes, we administered intravenous ^{127}I-iododexetimide to control C57Bl6 (n = 5) and KO mice (n = 5 except for M$_3$, where n = 3). The KO mice included M$_1$, M$_2$, M$_3$, M$_4$, and M$_5$ animals. For this experiment, mice received a 30 μg/kg dose of ^{127}I-iododexetimide (dose volume of 5 mL/kg of body weight) via the lateral tail vein and were sacrificed 40 min afterward.

Calculations, curve fitting, and graphics of the in vitro competitive binding experiments and ex vivo LC-MS/MS biodistribution studies were done using GraphPad software, version 4.0.

Storage Phosphor Imaging Experiments

To quantify the influence of acute and chronic release of acetylcholine and the effects of competitive binding to M$_1$R on ^{123}I-iododexetimide binding, phosphor storage imaging was conducted concurrently with acute and chronic administration of haloperidol and olanzapine. Haloperidol is a dopamine D$_2$ receptor blocker that leads to increased acetylcholine release (22) but has a low affinity for the mAchR M$_1$R (K$_i$ = 1,475 nM) (23). Olanzapine blocks the D$_2$ receptor but has a high affinity for M$_1$Rs (K$_i$ = 1.9 nM) (23). If ^{123}I-iododexetimide preferentially binds to M$_1$R, ^{123}I-iododexetimide binding will be influenced significantly by olanzapine but only marginally by haloperidol (indirectly by acetylcholine release, which may compete for binding). In total, 54 male Wistar rats (n = 8–10 per group) participated.

Drugs and Treatment Protocol. Two experiments were conducted, one to measure acute effects and one to assess the effects of chronic or subchronic administration of haloperidol and olanzapine on ^{123}I-iododexetimide binding. For the acute experiments, a single intraperitoneal injection of haloperidol (1 mg/kg of body weight), olanzapine (2.5 mg/kg of body weight), or placebo (saline) was administered. The chronic experiment lasted 2 wk, during which the animals were injected intraperitoneally twice a day with haloperidol (1 mg/kg), olanzapine (2.5 mg/kg), or placebo. Both haloperidol and olanzapine were dissolved in saline.

Storage Phosphor Imaging. One hour after drug injection in the acute group, and 24 h after the final injection in the chronic group, the animals were anesthetized via intraperitoneal administration of ketamine and xylazine (2:1) and injected with approximately 37 MBq of ^{123}I-iododexetimide (specific activity > 185 MBq/nmol; radiochemical purity > 95%) in the tail vein. The radiosynthesis was described earlier (24). This dose corresponds to a tracer dose rather than a pharmacologic dose; details and discussion are provided in the supplemental materials. Two hours after injection of the radiotracer, the anesthetized animals were sacrificed by cardiac puncture followed by dislocation of the spine. The
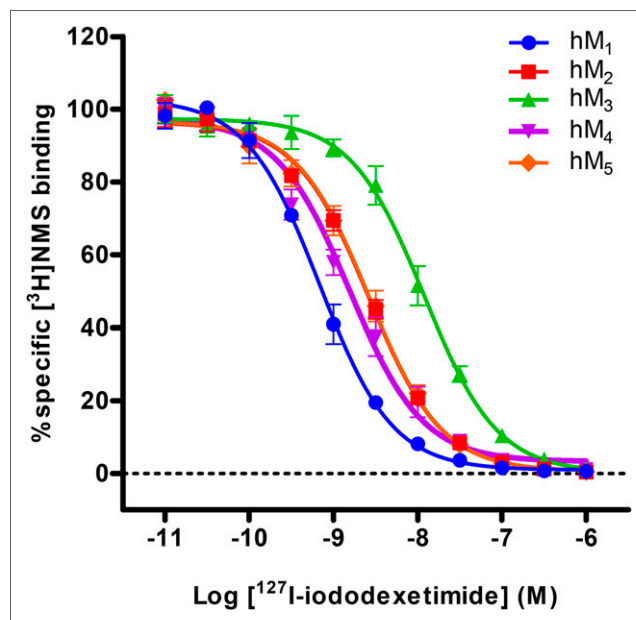

FIGURE 1. Displacement of ^{3}H-n-methylscopolamine (^{3}H-NMS) radioligand binding by ^{127}I-iododexetimide across mAchR subtypes M$_1$–M$_5$. Data points represent mean specific binding ± SEM from 3 independent experiments each containing 3 replicates.

TABLE 1
K_i Values for ^{127}I-Iododexetimide

mAchR subtype	K_i/pM	Selectivity ratio vs M_1
M_1	337	—
M_2	1,358	4.0
M_3	5,694	16.9
M_4	645	1.9
M_5	1,332	4.0

brains were quickly removed, frozen in nitrogen, and sectioned horizontally into 50-μm slices. Every fifth slice was placed on a phosphor imaging plate (Hypercassette; Amersham Biosciences) for 24 h (25). Subsequently, the plates were scanned at a 25-μm resolution with a 16-bit pixel depth using a Typhoon FLA 7000 phosphor imager (GE Healthcare) (26).

For analysis purposes, regions of interest (ROIs) were drawn for the M_1R-rich prefrontal cortex, hippocampus, and striatum (11,14). The cerebellum was chosen to assess nonspecific binding and was analyzed using AIDA (Automatic Image Data Analysis) image analysis as described earlier (27). Outcome values were ratios of ROI binding to cerebellum binding minus 1 (binding potential).

All storage phosphor imaging experiments were approved by the local Animal Welfare Committee at the Academic Medical Center in Amsterdam and were conducted according to Dutch regulations governing animal welfare and protection. All animal research conducted by Eli Lilly was in accordance with the National Research Council Guide under protocols approved by the Animal Care and Use Committee of Eli Lilly and Co.

Statistical Analyses

One-way ANOVA was performed to compare differences in frontal cortex/cerebellum binding potential between the conditions in the liquid chromatography mass spectroscopy studies in KO mice (M_1–M_5 KO and control mice). When there was a significant main effect, a post hoc analysis was performed using the Tukey multiple-comparison test.

For the storage phosphor imaging experiments, differences in striatal, prefrontal, and hippocampal muscarinic receptor binding ratios among

the 3 treatment conditions (olanzapine, haloperidol, and placebo treatment) were analyzed using 1-way multivariate ANOVA. Dependent variables were the separate ROI–cerebellum binding ratios. Treatment condition was the fixed factor. When there was a significant main effect, a post hoc analysis was performed. Statistical analyses for the storage phosphor imaging experiments were performed using the Statistical Package for the Social Sciences (SPSS, version 20.0; IBM).

RESULTS

In Vitro Competitive Binding Experiments

^{127}I-iododexetimide displayed a high affinity for M_1R (on average, 337 pM). The affinity for M_1R was 1.9–16.9 times higher than for the other 4 mAchRs (Fig. 1; Table 1). Further, in vitro profiling was performed using a GTPγ^{35}S binding assay to assess functional antagonism of ^{127}I-iododexetimide on acetylcholine-activated M_1R–M_5R (Fig. 2). ^{127}I-iododexetimide displayed an average IC_{50} of 31 nM for M_1R. The IC_{50} was 2.6–20.7 times higher than for the other muscarinic receptor subtypes (Fig. 2; Table 2).

LC-MS/MS Ex Vivo Characterization Experiments

Ex vivo characterization of ^{127}I-iododexetimide showed that binding was differentially distributed in wild-type rat brain, with a slow time course. ^{127}I-iododexetimide levels continued to increase in the frontal cortex and striatum over the 1-h period. Cerebellar and plasma levels of ^{127}I-iododexetimide began to wash out as early as 20 min after injection (Supplemental Fig. 1). ^{127}I-iododexetimide had a specific binding signal at all time points measured in rats (Supplemental Fig. 1). There was a significant main effect in the M_1 KO mice compared with controls; M_3, M_4, and M_5 KO mice ($P < 0.01$); and M_2 KO mice ($P < 0.001$) (Fig. 3.). Binding potential was significantly reduced in M_1 KO mice.

To validate specific binding of ^{127}I-iododexetimide to muscarinic receptors, groups of rats received doses of the $M_{1/4}$R agonist xanomeline (Fig. 4). Xanomeline reduced the levels of ^{127}I-iododexetimide in both rat frontal cortex and rat striatum in a dose-dependent manner; whereas no changes were observed in cerebellar and plasma ^{127}I-iododexetimide levels.

Storage Phosphor Imaging Experiments

One rat from the chronic experiment was excluded because of a binding ratio more than 2 SDs above the mean, resulting in a control group of 7 animals.

After a single administration of olanzapine, but not haloperidol, ex vivo ^{123}I-iododexetimide binding ratios significantly decreased compared with placebo-treated rats (Figs. 5 and 6; Table 3). A significant main effect of the treatment condition in the acute experiment was found for all 3 ROIs ($P < 0.001$), and post hoc analyses showed that the effect was driven by differences between olanzapine and both haloperidol ($P < 0.001$) and placebo ($P < 0.001$). No differences
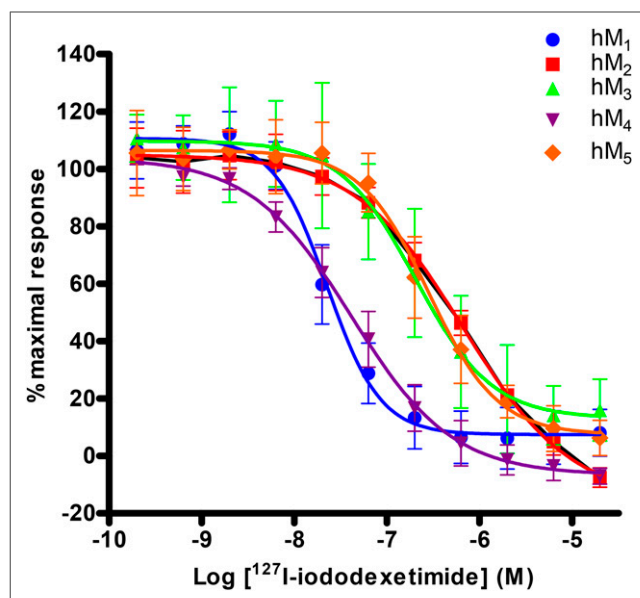

FIGURE 2. ^{127}I-iododexetimide in vitro muscarinic receptor selectivity profile by GTPγ^{35}S. Data are expressed as mean ± SEM from 3 separate experiments.

TABLE 2
IC_{50} Values for ^{127}I-Iododexetimide

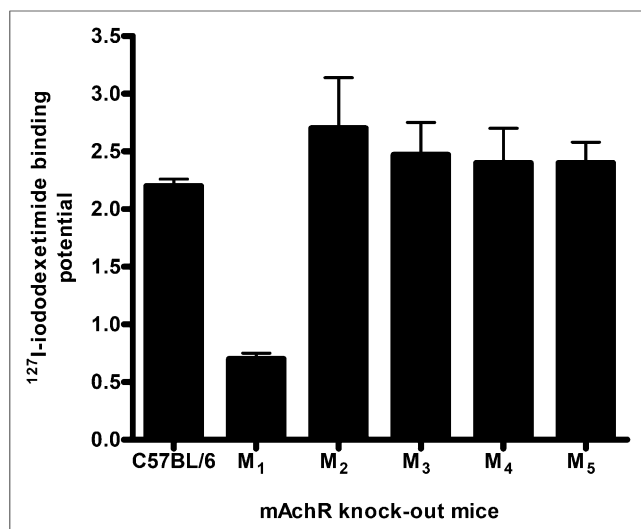| mAchR subtype	IC_{50}/nM	Selectivity ratio vs M_1
M_1	31	—
M_2	643	20.7
M_3	157	5.1
M_4	82	2.6
M_5	566	18.3

FIGURE 3. Validation of specific binding to M_1Rs of ^{127}I-iododexetimide in M_1–M_5 muscarinic receptor KO mice, 40 min after injection. Binding potential was calculated as specific binding in frontal cortex (total binding minus nonspecific binding) divided by nonspecific binding (measured in cerebellum).

were found between the haloperidol and placebo condition for any ROI (Table 3). After chronic administration of antipsychotics, no significant differences for treatment condition were found in any ROI ($P = 0.425$) (Supplemental Fig. 2).

t tests were performed to check for possible differences in M_1 binding between the acute and chronic treatment conditions for each ROI separately. After correction for multiple comparisons, the only significant differences were found in the olanzapine condition between acute and chronic administration in all 3 ROIs (Table 3).

DISCUSSION

The current study aimed to characterize ^{123}I-iododexetimide binding to mAchRs by studying in vitro selectivity, ex vivo ^{127}I-iododexetimide binding in rats and KO mice for the 5 different mAchR, the effects of an M_1 agonist on ex vivo ^{127}I-iododexetimide binding, and the effects of antipsychotics with high and low affinity for M_1R on ex vivo ^{123}I-iododexetimide binding in rats.

The in vitro experiments showed that ^{127}I-iododexetimide has a high affinity for M_1R and lower affinity for other mAchR subtypes (ranging from 1.9-fold to 16.9-fold). In the rat study, ^{127}I-iododexetimide levels continued to increase in the frontal cortex and striatum over the period measured (1 h), and ^{127}I-iododexetimide exhibited specific uptake in the frontal cortex and striatum. ^{127}I-iododexetimide binding was significantly reduced in the M_1 KO mice, suggesting that most of the specific ^{127}I-iododexetimide binding is attributed to M_1R in vivo. Furthermore, the agonist xanomeline dose-dependently decreased ^{127}I-iododexetimide binding in M_1-rich brain areas. Finally, as opposed to haloperidol and placebo, ^{123}I-iododexetimide binding was reduced significantly after a single administration of the antipsychotic drug olanzapine, which displays high affinity for M_1R. No differences in ^{123}I-iododexetimide binding were found among the 3 treatment conditions after chronic administration, from which one may infer no significant receptor up- or downregulation after chronic administration of olanzapine or haloperidol.

For two decades, ^{123}I-iododexetimide has been used as a radiopharmaceutical to assess mAchR binding in neuropsychiatric disorders in humans (*24,28,29*). In the 1980s, ^{123}I-iododexetimide was

characterized by in vitro experiments, but the binding assays were performed on tissue homogenates that express all mAchRs (*17*). Later, techniques were developed to assess binding to subtypes of mAchR. To our knowledge, the current study is the first to characterize the binding selectivity and affinity of ^{123}I-iododexetimide for each individual mAchR subtype. The radioligand binding experiments in the current study showed that ^{127}I-iododexetimide displayed a very high affinity for the human M_1R (mean K_i of 337 pM) but also high affinity for the other 4 muscarinic receptor subtypes. This was confirmed by the GTPγ^{35}S assay, which showed that in human M_1R membranes, ^{127}I-iododexetimide had the greatest potency to reduce an agonist response evoked by acetylcholine (mean IC_{50} of 31 nM). This finding is also in line with earlier studies showing that ^{127}I-iododexetimide is an antagonist (*17*). This information is crucial to the interpretation of results from ^{123}I-iododexetimide SPECT imaging studies because agonists and antagonists may recognize different populations of the same receptor subtype (e.g., high- and low-affinity states (*30*)). Because antagonist radiotracers may bind to receptors in the high- as well as the low-affinity state, ^{123}I-iododexetimide SPECT may be used to assess overall muscarinic M_1R availability, whereas agonist tracers (which may bind predominantly to the receptor in its high-affinity state) may be more sensitive to detecting changes in acetylcholine concentrations.

Regarding selectivity, the radioligand binding experiments showed that ^{127}I-iododexetimide has a 2-fold selectivity for human M_1R over human M_4R and a 4-fold selectivity over human M_2R and human M_5R. This preference for human M_1R is also shown in the GTPγ^{35}S assay, which shows a 2.6-fold selectivity over human M_4R, a 5-fold selectivity over human M_4R, and around a 20-fold selectivity over human M_2R and human M_5R. The biodistribution studies in both rats and wild-type mice showed that the highest binding of ^{127}I-iododexetimide was in the frontal cortex and striatum, areas with high M_1R densities. So, specific binding seems predominantly attributable to M_1R, as is in line with our in vitro results. This selectivity for M_1R is also confirmed by the experiments
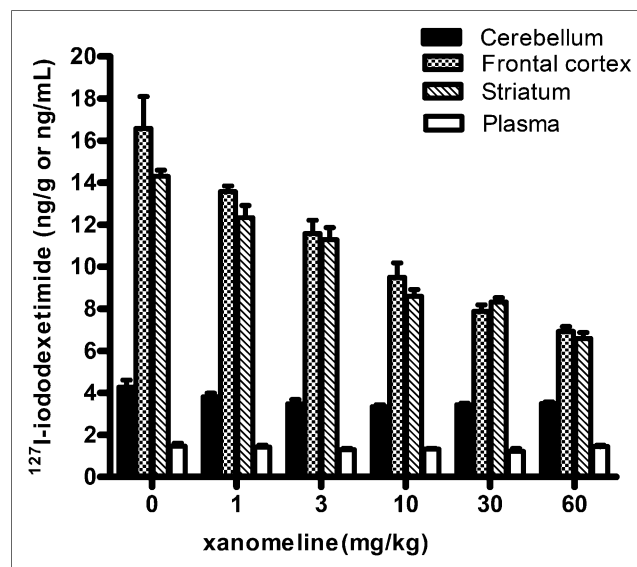

FIGURE 4. Distribution of ^{127}I-iododexetimide in cerebellum, frontal cortex, striatum, and plasma ($n = 3$–4) in response to dose increase of agonist xanomeline (1–60 mg/kg) 40 min after injection of ^{127}I-iododexetimide. Mice were pretreated with vehicle (0) or xanomeline 30 min before injection of radiotracer. Data points represent mean specific binding ± SEM.
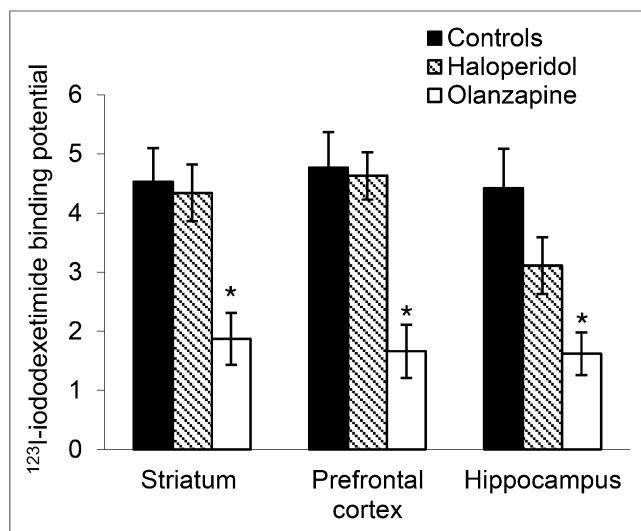
FIGURE 5. Binding potential of ^{123}I-iododexetimide in ROIs of striatum, prefrontal cortex, and hippocampus. Binding potential was calculated as specific binding (total binding minus nonspecific binding) in ROI divided by nonspecific binding (measured in cerebellum). ^{123}I-iododexetimide binding was measured 2 h after intravenous injection of ^{123}I-iododexetimide. Rats (n = 10/group) were pretreated with 1 dose of saline, haloperidol (1 mg/kg), or olanzapine (2.5 mg/kg) 1 h before injection with radiotracer. *Statistically significantly lower as compared with control group.

on KO mice in which only the KO of the M_1 gene caused a significant decrease in [127]I-iododexetimide binding in M_1-rich brain areas, compared with the wild-type and other mAchR subtype KO mice.

In the rat, acute administration of the M_1/M_4 agonist xanomeline or the antipsychotic drug olanzapine, which is known to also block M_1Rs, reduced [127]I-iododexetimide and ^{123}I-iododexetimide binding potential, respectively, whereas the relatively dopamine D_2–selective antipsychotic haloperidol (31) did not affect ^{123}I-iododexetimide binding significantly. This finding confirms that the in vivo binding of ^{123}I-iododexetimide reflects binding to M_1R; this finding also may indicate that caution is needed in applying ^{123}I-iododexetimide in vivo to humans who are treated with olanzapine or other antipsychotics with a muscarinic or antimuscarinic profile, such as clozapine. Indeed, in a previous ^{123}I-iododexetimide SPECT study, we showed that schizophrenic patients stabilized on olanzapine had significantly lower ^{123}I-iododexetimide binding in the frontal cortex and striatum than patients stabilized on risperidone (24).

As expected, acute treatment with olanzapine did induce lower ^{123}I-iododexetimide binding, representing occupancy of M_1Rs by the drug. Interestingly, acute or chronic treatment with haloperidol did not affect ^{123}I-iododexetimide binding significantly, possibly indicating that it is not necessary to discontinue use of haloperidol if one is interested in studying M_1R expression. Although Damsma et al. (22) found increased acetylcholine release after administration of haloperidol in rats, these changes in acetylcholine release evoked by haloperidol may be too small to compete with ^{123}I-iododexetimide binding in vivo. Also, no effects of chronic administration of olanzapine were found on ^{123}I-iododexetimide binding potential after a washout period of 24 h, suggesting that only a relatively short washout period of this drug is necessary if one is interested in studying M_1R expression.

The biodistribution study on rats using [127]I-iododexetimide and liquid chromatography–mass spectroscopy analysis showed that uptake of [127]I-iododexetimide was highest in the frontal and striatal tissues, with low specific binding in the cerebellum, a brain area with low levels of M_1Rs, similar to the present findings with ^{123}I-iododexetimide. An advantage of storage phosphor imaging is that the measurement is not influenced by metabolites of ^{123}I-iododexetimide that are formed after injection. Both techniques showed high binding potential, suggesting that metabolites of ^{123}I-iododexetimide formed in the brain are not a major issue in quantification of ^{123}I-iododexetimide binding in clinical practice. As early as 1 h after injection of ^{123}I-iododexetimide, the specific-to-nonspecific binding ratios were already 3. A study by Wilson et al. (17) equally showed a binding ratio of 3–4 at 1 h after injection in mice; our results are thus congruent with this ratio in the wild-type mice and the rat, advocating ^{123}I-iododexetimide as an accurate tool for human applications.

Besides ^{123}I-iododexetimide, other antagonist radiopharmaceuticals for muscarinic receptors (e.g., ^{18}F-FP-TZTP [3-(4-(3-^{18}F-fluoropropylthio)-1,2,5-thiadiazol-3-yl)-1-methyl-1,2,5,6-tetrahydropyridine] or ^{123}I-QNB [(R,R)[123]I-iodoquinuclidinylbenzilate]) are available, but none of them is known to bind selectively to M_1R. ^{123}I-iododexetimide has some advantages over ^{123}I-QNB; ^{123}I-iododexetimide can be synthesized with high specific activity more easily and has a much higher synthetic yield, making it a preferable radioligand to assess M_1R binding. In humans, ^{123}I-iododexetimide showed a high nondisplaceable binding potential (>3 at 6 h after injection) (19). The current study provides the pharmacodynamic evidence that ^{123}I-iododexetimide in humans most likely evaluates predominantly the availability of M_1R in the frontal cortex, striatum, and hippocampus (32). With respect to cognition, these regions are highly pertinent, and ^{123}I-iododexetimide SPECT may be an important means to assess M_1R expression in vivo.

Although [127]I-iododexetimide had the highest affinity for M_1R, the binding to the other 4 mAchRs was relatively high (Fig. 1). More specifically, the affinity of [127]I-iododexetimide was in the picomolar range for both the M_1 and M_4R, although there was some selectivity (2 times higher binding to M_1R). Interestingly, the studies on the KO mice showed that only KO of M_1R reduced the [127]I-iododexetimide binding significantly. However, studies on rats showed that the expression of M_1R (B_{max}) was 3–5 times higher in the M_1R-rich frontal cortex (33). Since the binding potential is influenced by B_{max}, as well as by the affinity of the radiotracer for a given receptor, it is likely that the combination of a higher affinity and a higher B_{max} may lead to preferential binding of ^{123}I-iododexetimide to M_1R.

When the present results are interpreted, some limitations have to be considered. Because the time of animal sacrifice was different in all experiments, direct comparison of results is difficult. In addition, the duration of antipsychotic treatment in the chronic rat experiment was only 2 wk. Therefore, we cannot rule out an influence of antipsychotics such as haloperidol on M_1R binding after a longer
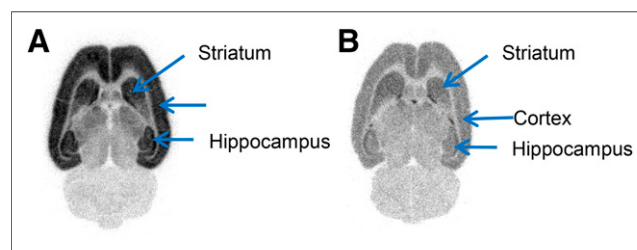

FIGURE 6. Intense ^{123}I-iododexetimide binding (2 h after injection of radiotracer) in cortical brain areas, striatum, and hippocampus in placebo-treated rat (A) and much lower binding in these brain areas (B) after acute olanzapine treatment. Rats (n = 10) were pretreated with 1 dose of saline or olanzapine (2.5 mg/kg) 1 h before injection with radiotracer.

TABLE 3
Differences Between Acute and Chronic
^{123}I-Iododexetimide Binding Potential for All ROIs

Region	Acute		Chronic			
	Mean	SD	Mean	SD	t	P
Striatum						
Haloperidol	4.34	0.48	3.78	0.46	2.49	0.024
Olanzapine	1.87	0.44	3.70	0.74	−6.41	<0.001*
Placebo	4.53	0.57	3.79	0.87	2.14	0.049
Prefrontal cortex						
Haloperidol	4.63	0.40	3.99	0.47	3.13	0.007
Olanzapine	1.66	0.45	4.26	0.80	−8.22	<0.001*
Placebo	4.77	0.60	4.21	0.94	1.51	0.152
Hippocampus						
Haloperidol	3.11	0.48	3.92	0.67	0.70	0.492
Olanzapine	1.62	0.36	3.70	0.84	−7.03	<0.001*
Placebo	4.42	0.67	4.02	1.13	0.93	0.369

*Significant after correction for multiple comparisons.

treatment period. Finally, the rats were anesthetized in the storage phosphor imaging studies, and we consequently cannot exclude a systematic effect on ^{123}I-iododexetimide binding.

CONCLUSION

In vivo binding of ^{123}I-iododexetimide predominantly reflects binding to M_1R. Therefore, ^{123}I-iododexetimide may be an accurate ligand for in vivo measuring of M_1R expression in humans. Because M_1R is associated with cognitive functioning, imaging of M_1R may be of great importance in, for example, selecting patients and monitoring the effects of new treatments.

DISCLOSURE

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