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Demonstration of a reduction in muscarinic receptor binding in early Alzheimer's disease using iodine-123 dextetimide single-photon emission tomography

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Abstract. Decreased muscarinic receptor binding has been suggested in single-photon emission tomography (SPET) studies of Alzheimer's disease. However, it remains unclear whether these changes are present in mildly demented patients, and the role of cortical atrophy in receptor binding assessment has not been investigated. We studied muscarinic receptor binding normalized to neostriatum with SPET using [¹²³I]4-iododexetimide in five mildly affected patients with probable Alzheimer's disease and in five age-matched control subjects. Region of interest (ROI) analysis was performed in a consensus procedure blind to clinical diagnosis using matched magnetic resonance (MRI) images. Cortical atrophy was assessed by calculating percentages of cerebrospinal fluid in each ROI. An observer study with three observers was conducted to validate this method. Alzheimer patients showed statistically significantly less [¹²³I]4-iododexetimide binding in left temporal and right temporo-parietal cortex compared with controls, independent of age, sex and cortical atrophy. Mean intra-observer variability was 3.6% and inter-observer results showed consistent differences in [¹²³I]4-iododexetimide binding between observers. However, differences between patients and controls were comparable among observers and statistically significant in the same regions as in the consensus procedure. Using an MRI-SPET matching technique, we conclude that [¹²³I]4-iododexetimide binding is reduced in patients with mild probable Alzheimer's disease in areas of temporal and temporo-parietal cortex.

Key words: Dextetimide – Single-photon emission tomography – Alzheimer's disease – Muscarinic receptor imaging – Cortical atrophy

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Introduction

Several ligands have been developed for in vivo single-photon emission tomography (SPET) imaging of muscarinic receptors in Alzheimer's disease (AD), including 3-quinuclidinyl-4-[¹²³I]iodobenzilate (IQNB) and, more recently, [¹²³I]4-iododexetimide (¹²³IDEX). ¹²³IDEX was developed as a high-affinity, non-selective muscarinic receptor antagonist. Müller-Gärtner and colleagues [1] reported in a study of four young normal volunteers that it is an attractive ligand owing to its high ratio of specific to non-specific binding. It seems likely that ¹²³IDEX binds to several subtypes in vivo, since binding was reported in neostriatum and neocortex, where M₁ receptors predominate, as well as in thalamus and cerebellum, where M₂ receptors prevail [1].

Alzheimer patients reportedly have bilateral reductions of IQNB receptor binding in posterior temporal cortex [2, 3], and in other cortical areas in some patients [3]. However, the possibility that these results are explained by cortical atrophy cannot be ruled out [2], and reduction of receptor density may take place only at a very late stage of AD [3]. We therefore designed a muscarinic receptor binding study in AD patients and normal controls with ¹²³IDEX to provide answers to the following questions. First, is muscarinic receptor binding reduced in selected patients with mild AD? Preliminary results of one SPET study using ¹²³IDEX suggest that this might be the case for temporo-parietal cortex [4]. Muscarinic receptor decrease in an early stage of the disease may be clinically important because of a possible relationship with failure of cholinomimetic treatment. Second, if there are reductions in muscarinic receptor binding, can these results be explained by differences in cortical atrophy between mild AD patients and controls?

Magnetic resonance imaging (MRI) studies were performed in all subjects, and we developed a new method for regions of interest (ROI) analysis with matched SPET and MRI images. This method enabled us to calculate the percentage of cerebrospinal fluid (CSF) space in each ROI as an estimate of the degree of cortical atrophy. Results of a validation study of the ROI analysis in an inter- and intra-observer study with three observers are presented.

Materials and methods

Subjects. Five patients with probable AD according to NINCDS-ADRDA criteria [5], recruited from the Memory Outpatient Clinic at the Academic Medical Center, participated in this study. All patients were classified as having mild dementia severity according to CAMDEX criteria [6], and both patients and family members provided informed consent after full disclosure of potential risks and benefits. Approval was obtained from the Medical Ethics Committee of the Academic Medical Center. Patients had not used any central nervous system active medication for at least 4 weeks prior to study. Cognitive function was evaluated with the Cambridge Cognitive Examination (CAMCOG), the cognitive test that is part of the CAMDEX-N [7]; the Mini-Mental State Examination (MMSE) [8] is part of the CAMCOG. The CAMCOG sum score has a high test-retest reliability and includes items assessing orientation, language, praxis, attention, abstract thinking, perception and calculation [9]. Five controls of the same age range were selected as spouses of patients or volunteers from advertisements. Controls with memory complaints or with abnormalities on neurological examination were excluded.

SPET. For each subject a brain ^{123}I DEX SPET was made with the Strichmann Medical Equipment 810x system. ^{123}I labelling was performed by Cygne B.V. (Technical University Eindhoven, The Netherlands). A single dose of ^{123}I DEX was used to image muscarinic receptors in the human brain. The administered intravenous dose was 185 MBq with a specific activity of 222 MBq/nmol, which equals 0.83 nmol or 1 μg of ^{123}I DEX. Multi-slice SPET image acquisition was performed 8 h after injection. All subjects

took potassium iodide orally in order to block thyroid uptake of free radioactive iodine.

The Strichman camera consists of 12 focal-point detectors. Scans were made slice after slice by moving out the patient bed automatically 0.5 cm every 150 s, parallel with and starting at the orbitomeatal line. The energy window was set at 135–190 keV. Data acquisition took place in a 64×64 matrix. During acquisition of one slice all detectors moved in an axial and a transaxial direction such that the focal points of the detectors scanned the complete volume within that slice. Reconstruction of the acquisition data was done using the heuristic algorithm according to the manufacturer's protocol package [10]. Each reconstructed scan consisted of 15–21 slices with a pixel size of $0.32 \times 0.32 \text{ cm}^2$ and a slice to slice distance of 0.5 cm. The transaxial resolution of the Strichman camera is 7.6 mm full-width at half-maximum of a line source in the air, while the axial resolution is 13.5 mm.

MRI. T1-weighted spin-echo images were obtained in both patients and controls with a Siemens Magnetom 63SP/4000 scanner (TR=610 ms, TE=14 ms). Each MRI scan consisted of 19 transaxial slices, with a pixel size of $0.09 \times 0.09 \text{ cm}^2$ and a slice to slice distance of 6.5 mm (center to center 5 mm, slice gap 1.5 mm). Care was taken that the MRI volume enclosed the corresponding SPET scan.

SPET and MRI matching procedure. SPET and MRI images were registered using chamfer matching. This method has been successfully used previously in registering CT-CT, CT-MRI and CT-SPET [11].

Chamfer matching does not use external fiducial markers but registers on intrinsic image information. In both modalities corresponding structures are detected, followed by an efficient minimization of the average distance between the structures. Since ^{123}I DEX is reasonably homogeneously distributed over the surface of the brain, binary segmentation can be used to detect the brain in SPET with sufficient accuracy. In MRI the brain surface is detected using morphological filters. For this study the chamfer method achieved an accuracy of approximately 0.42 cm, which is within the SPET resolution of 0.7 cm.

Regions of interest. ROIs of frontal, temporal, temporo-parietal and parietal cortex were drawn by hand on the MRI image blindly

Fig. 1. ROI analysis with matched MRI (left) and SPET (right) images in a normal control subject, shown for frontal (F), temporal (T), temporo-parietal (TP) and striatal (S) regions

to clinical diagnosis, in consensus between two observers with reference to an anatomical atlas (Fig. 1) [12]. Strichman Medical Units (SMUs counts $s^{-1} mm^{-2}$) were calculated in corresponding ROIs on the matched SPET images (Fig. 1). The temporo-parietal region was defined in the transition area of the temporal and parietal cortex, where no reliable differentiation between these two areas could be made [13]. For each cortical area two image slices were included in the ROI analysis.

In the study by Müller-Gärtner and colleagues using ^{123}I DEX, immediately after the injection peak, activity in the cerebellum decreased significantly [1]. Activity in neocortex, neostriatum, and thalamus increased over 7–12 h after injection and activity was highest in neostriatum, followed in rank order by neocortex, thalamus and cerebellum [1]. Since the neostriatum is not affected in AD [14], this area was chosen as a reference region. The left and right regions corresponding to the anatomical area of the neostriatum [12] (Fig. 1) were drawn by hand in one slice. Then, the average value in counts per pixel of these left and right regions was used as a reference value to normalize values of cortical ^{123}I DEX binding. Normalized ^{123}I DEX binding was defined as the average counts per pixel within an ROI divided by that of the neostriatal reference region using the following formula: left or right ^{123}I DEX binding in cortical ROIs in average counts per pixel/mean of left and right neostriatal ROI in average counts per pixel.

An observer study was performed according to the following procedure. To assess inter-observer variability, three observers drew ROIs in all subjects, independently from each other and blind to clinical diagnosis. ROIs were drawn in frontal, parietal, temporo-parietal and temporal cortical areas and included one slice for each ROI. The reference region was drawn as in the consensus procedure, in left and right neostriatum and in one slice. To assess intra-observer variability, each observer repeated the drawing of the ROIs twice; thus altogether three sessions per observer for each ROI were available for analysis. These sessions were separated from each other by at least one week.

Assessment of CSF spaces within ROIs. The percentage of CSF within a given ROI was calculated on the MRI image. Histogram analysis was used on all MRI studies to define the lower pixel threshold for healthy brain tissue (LTH). To determine the optimal value of LTH, the information of an ROI from MRI images of brain tissue and CSF spaces was used to produce one histogram. In this histogram one peak can be seen for the MRI-pixel value for brain tissue and one peak for CSF. The LTH was defined as the lowest MRI-pixel value in between these two peaks. Since all studies were acquired with the same MRI system and with the same parameters (TR=610 ms, TE=14 ms), a standard LTH of 288 could be defined for all MRI studies. For each ROI the number of pixels with a value below LTH was divided by the total number of pixels to obtain an estimate of the percentage of CSF within that ROI. Using this method and the aforementioned LTH value for all MRI studies, we verified the validity of the method by establishing that the differentiation of brain tissue and CSF in both patients and controls was in agreement with the visual judgement of an expert.

Statistical analysis. Differences in normalized ^{123}I DEX binding between AD patients and normal controls were analysed with multiple linear regression analysis, adjusted for age, sex and percentage of CSF space in a given ROI (BMDP 1R) [15]. For the observer study, intra-observer variability was calculated in percentage normalized ^{123}I DEX binding for three consecutive sessions of ROI analysis. Inter-observer variability was assessed by

determining absolute differences between observers in a given ROI. Further, the observer versus group interaction was used as a measure to determine whether the detected differences between AD patients and controls varied between observers [16]. The intraclass correlation coefficient serves as a measure of how much variability of differences between patients and controls can be attributed to variability among different observers [16]. The coefficient calculates the relation between the variability between patients and controls on the one hand, and both the variability of the difference between patients and controls and the variability attributable to the different observers on the other hand. A high coefficient indicates that observer variability does not play a role in the detected differences between patients and controls. The observer agreement analysis was performed with BMDP 8V [17].

Results

Some characteristics of the study population are presented in Table 1. There were no differences in age between AD patients and controls, but MMSE and CAMCOG scores were significantly lower in AD patients ($P<0.01$). All patients were classified as mildly demented according to CAMDEX criteria [6].

Assessments of regional cortical normalized ^{123}I DEX binding are shown in Table 2. ^{123}I DEX binding was significantly lower in AD patients, compared with normal controls, in left temporal cortex ($P<0.01$) and right temporo-parietal cortex ($P<0.05$), independent of age, sex and percentage of CSF space in given ROIs. Analysis of regional ^{123}I DEX binding with and without adjustment for percentage of CSF space revealed similar results. No statistically significant differences between patients and controls were observed for percentages of regional CSF space in ROIs (Table 2).

Results of the observer study are presented in Table 3. Significant differences in normalized [^{123}I]DEX binding between AD patients and controls were found for the three observers in left temporal and right temporo-parietal cortex, the same areas that were statistically significant in the analysis with consensus agreement. The magnitude of the observed differences between patients and controls were similar to those found in the consensus

Table 1. Subject characteristics

	Normal controls (n=5)	Alzheimer patients (n=5)
Age (years)	76.6±6.1	76.4±3.4
Sex (men/women)	3/2	2/3
Mini-Mental State Examination [8]	28.4±1.3	18.6±1.8*
CAMCOG ^a	98.8±3.1	66.4±7.1*

Data are presented as mean±SD

* Significantly different from controls at $P<0.01$

^a Cognitive test from the Cambridge Examination for Mental Disorders of the Elderly (CAMDEX) [7]

Table 2. ^{123}I IDEX receptor binding relative to neostriatum ($\pm\text{SD}$) and percentage of CSF space in ROIs in five normal controls and in five patients with AD

ROI	^{123}I IDEX receptor binding relative to neostriatum		Percentage of CSF space in ROIs	
	Normal controls	Alzheimer patients	Normal controls	Alzheimer patients
Frontal right	0.87 $\pm$ 0.05	0.78 $\pm$ 0.09	22.1 $\pm$ 8.5	18.7 $\pm$ 15.2
Frontal left	0.89 $\pm$ 0.05	0.80 $\pm$ 0.09	23.4 $\pm$ 15.7	17.2 $\pm$ 10.8
Temporal right	0.99 $\pm$ 0.08	0.94 $\pm$ 0.14	21.2 $\pm$ 9.6	22.8 $\pm$ 16.7
Temporal left	1.00 $\pm$ 0.04	0.90 $\pm$ 0.04**	22.0 $\pm$ 5.6	27.2 $\pm$ 15.2
Temporo-parietal right	1.02 $\pm$ 0.03	0.90 $\pm$ 0.09*	16.4 $\pm$ 14.3	12.5 $\pm$ 9.7
Temporo-parietal left	0.98 $\pm$ 0.07	0.93 $\pm$ 0.07	15.7 $\pm$ 12.8	19.1 $\pm$ 13.7
Parietal right	0.83 $\pm$ 0.09	0.85 $\pm$ 0.17	21.8 $\pm$ 5.9	16.5 $\pm$ 9.6
Parietal left	0.80 $\pm$ 0.09	0.81 $\pm$ 0.03	19.9 $\pm$ 4.3	18.1 $\pm$ 6.6

* $P<0.05$, ** $P<0.01$ **Table 3.** ^{123}I IDEX receptor binding relative to neostriatum as assessed by three observers in normal controls and in patients with AD

ROI	Observer 1		Observer 2		Observer 3		<i>P</i> value group effect
	Normal controls	Alzheimer patients	Normal controls	Alzheimer patients	Normal controls	Alzheimer patients	
Frontal right	0.77	0.77	0.89	0.83	0.84	0.80	0.54
Frontal left	0.85	0.80	0.93	0.83	0.89	0.80	0.15
Temporal right	0.96	0.95	1.03	0.97	0.91	0.89	0.64
Temporal left	1.00	0.90	1.03	0.94	0.98	0.87	0.02
Temporo-parietal right	0.98	0.89	1.00	0.90	0.97	0.85	0.03
Temporo-parietal left	0.95	0.87	0.98	0.93	0.94	0.85	0.09
Parietal right	0.90	0.90	0.93	0.93	0.86	0.87	0.96
Parietal left	0.87	0.89	0.89	0.84	0.85	0.84	0.93

Table 4. Observer variability for matched ^{123}I IDEX SPET and MRI analysis

ROI	Intra-observer	Inter-observer	Observer versus group (Alzheimer and normal control) effects	
	Mean variability (%)	Observer effect	Observer-group interaction (<i>P</i> value)	Intra-class correlation coefficient
Frontal right	4.1	$P<0.01$	0.24	0.82
Frontal left	3.1	$P<0.01$	0.09	0.87
Temporal right	4.9	$P<0.01$	0.54	0.83
Temporal left	3.6	$P=0.01$	0.67	0.71
Temporo-parietal right	3.6	$P=0.02$	0.65	0.75
Temporo-parietal left	3.5	$P<0.01$	0.20	0.72
Parietal right	3.1	$P<0.01$	0.96	0.96
Parietal left	3.2	$P<0.01$	0.45	0.81

agreement assessment for all three observers. Mean intra-observer variability for the three observers and for three respective measurements was 3.6% (Table 4). Analysis of inter-observer variability showed significant differences between observers in all cortical regions (Ta-

ble 4), indicating that one observer had consistently higher or lower normalized ^{123}I IDEX binding values than other observers. However, there were no differences between observers in the detection of differences between AD patients and controls. Indeed, the observer-group in-

teraction was not statistically significant in any of the regions analysed (Table 4), showing that differences found between groups were comparable for all three observers. Furthermore, the variability of differences between patients and controls in normalized ^{123}I DEX binding that could be attributed to observer variability was very small, as evidenced by high intra-class correlation coefficients in all regions (Table 4).

Discussion

We studied normalized [^{123}I]DEX binding in a selected group of mildly demented AD patients, compared with control subjects. ROI analysis was performed according to a newly developed procedure with matched SPET and MRI images. AD patients showed statistically significantly lower normalized ^{123}I DEX binding in the left temporal and right temporo-parietal regions. No differences between patients and controls were observed for regional percentages of CSF space in different ROIs. Although consistent differences between observers were evident, our observer study showed that the ability to detect differences between patients and controls was similar for all observers, compared with consensus ROI analysis. In this observer analysis, normalized ^{123}I DEX binding values were also statistically significantly lower in AD patients in the left temporal and right temporo-parietal areas for all three observers.

Few previous studies have investigated the *in vivo* distribution of muscarinic cholinergic receptors in AD. Weinberger et al. studied IQNB binding in 12 mildly to moderately affected AD patients and found a significant reduction bilaterally in posterior temporal cortex compared with controls [2]. Wyper et al. studied IQNB binding in eight AD patients ranging from mild to severe dementia and in four control subjects. These authors concluded that a major reduction in postsynaptic receptor density is evident only in the very late stages of AD, based on analysed patterns of muscarinic receptor binding relative to regional cerebral blood flow. Our results demonstrate that decreased muscarinic receptor binding in posterior cortical areas is already evident at an early stage of the disease. This finding is supported by preliminary results of a SPET study using ^{123}I DEX as a tracer in mildly affected AD patients [4].

The degree of cortical atrophy is greater in AD than in normal aging [18–20]. Therefore, differences in CSF spaces in ROIs between AD patients and controls related to cortical atrophy could affect measurements of ^{123}I DEX binding. In particular, the limited resolution of ^{123}I DEX SPET imaging raises concerns over this potential measurement error, suggesting the necessity for correction with indicators of cortical atrophy. For this purpose, we developed a method with matched SPET and MRI images, and we found that the percentages of CSF space in each ROI were not different in AD patients and controls. Therefore, our assessment of differences in

^{123}I DEX binding between patients and controls is not affected by measurement errors related to cortical atrophy. Of course, this does not mean that cortical atrophy in AD patients was not different from normal controls, since we performed no measurements of total, ventricle or extraventricular CSF spaces on MRI images. Our findings are in agreement with a recent fluorine-18 fluorodeoxyglucose positron emission tomography study showing no significant differences in percent increase in activity after partial volume correction between AD patients and control subjects [21].

It is unlikely that our results obtained with ^{123}I DEX binding are dependent on regional cerebral blood flow (rCBF) for several reasons. Firstly, it is suggested that ^{123}I DEX meets criteria for specific binding to muscarinic receptors in humans *in vivo* because ^{123}I DEX binding activity correlates with muscarinic receptor concentrations from human brain tissue [1]. Secondly, there was a dissociation of the distribution of ligand retention between rCBF assessed with hexamethylpropylene amine oxime (HMPAO) and ^{123}I DEX SPET [1]. In control subjects the lowest ^{123}I DEX binding was observed in areas with maximum blood flow, including cerebellum and thalamus. Similar results were obtained in patients with dementia who were studied with IQNB, there being no retention in cerebellum and low retention in thalamus [3, 22]. Thirdly, we performed SPET scans 8 h after injection, at which time there is a plateau phase. Therefore, it is unlikely that blood flow mediates receptor binding at this time. Further, studies of rCBF using HMPAO were not performed in our subjects for confirmation of the diagnosis of AD. It has recently been suggested that rCBF SPET cannot make a significant contribution to the diagnosis of AD with NINCDS-ADRDA criteria [23]. Indeed, although some authors suggest a high overall diagnostic accuracy of HMPAO SPET [24], others report poorer test characteristics [13, 25].

The observer study showed that differences between patients and controls in ^{123}I DEX binding could not be attributed to variations between observers with the new method of ROI analysis. Indeed, the lower normalized [^{123}I]DEX binding in AD patients could be reproduced by three observers in the same cortical areas. The intra-observer variability in this study is low, with a mean of 3.6%. We found consistent differences between observers but these are not of major concern when the ability to detect differences between groups is similar across observers. This notion was supported by the statistical analysis showing that differences between groups were not affected by an observer effect. However, the consistent differences between observers may well be explained by the size, and especially the width, of the ROIs. Due to the limited resolution of MRI and SPET images, the matched SPET ROI may be positioned somewhat outside the cortical margin, resulting in lower ROI values, and large MRI ROIs would increase this tendency. A limitation of the method at present is that ROIs between observers cannot be readily compared be-

cause of these differences. Further improvement of the MRI-SPET matching procedure and efforts to reduce the inter-observer variability are therefore needed.

Earlier neuroreceptor studies in post-mortem AD brain tissue showed unchanged [26, 27], increased [28, 29] or decreased [30, 31] muscarinic receptor binding in different brain regions [32]. At least five different muscarinic receptor subtypes have been identified [33] and more recent evidence suggests alterations of these specific receptor subtypes and changes in signal transduction. Changes in M1 receptors, involved in the postsynaptic neurotransmission, are suggested by decreased immunoreactivity in comparison with control subjects [34] and by defective signal transduction [35] in the presence of normal levels of M1 receptors assessed by radioligand binding [34]. In addition, both decreased M2 receptor immunoreactivity and loss of M2 binding sites located at the presynaptic cholinergic terminals have been observed, while the M4 receptor shows evidence of up-regulation [34]. In another study, reduction of postsynaptic muscarinic receptor response was suggested by functional impairment of the receptor-G-protein complex, as evidenced by impaired phosphoinositide hydrolysis in AD brain [36].

As reported by Müller-Gärtner and associates, ^{123}I DEX has the potential to measure small changes in muscarinic receptors in vivo with SPET and accumulates predominantly in frontal, temporal, parietal and occipital cortex and in neostriatum [1]. Although this ligand shows specific binding to muscarinic receptors in vivo, it does not show any selectivity regarding different muscarinic receptor subtypes. Both biochemical evidence and results from in vivo muscarinic receptor imaging with SPET support the concept that these receptors are decreased in AD. The limited statistical power of our study probably explains why differences in normalized [^{123}I]DEX binding were observed in only left temporal and right temporo-parietal regions. Further studies with SPET using receptor-selective ligands are needed to obtain more detailed information about these in vivo muscarinic receptor changes.

Cholinergic replacement therapy in AD is dependent on the functional integrity of acetylcholine receptors. Therapeutic approaches include inhibition of the enzyme acetylcholinesterase, which degrades acetylcholine, and stimulation with agonists of the receptors situated postsynaptically to the degenerating cortical projections [37, 38]. Currently efforts are being made to develop centrally acting selective muscarinic agonists, acting at the M1 or M4 sites [39]. Clinical trials with tacrine, a cholinesterase inhibitor, have shown that this agent may confer symptomatic benefit to some Alzheimer patients [40–43]. Attempts to determine which patients may respond to treatment with tacrine have not been successful, however. A possible response to cholinergic replacement therapy may be related to reductions in muscarinic receptors or impairment in signal transduction [44, 45]. Therefore, it is tempting to speculate, based on the results of our study, that regional cerebral ^{123}I DEX binding

may represent a useful basis on which to select patients who will respond to such therapeutic interventions. However, since ^{123}I DEX binding is not selective for muscarinic receptor subtypes, it is more likely that selective ligands are candidates for treatment selection. ROI analysis is critical when large control groups are used for this purpose. Assessment of degree of cognitive impairment is also important in this regard, and overall CAMCOG scores provide only a rough measure of decline in cognitive function in the absence of detailed neuropsychological evaluation.

In conclusion, using a newly developed method with SPET-MRI matching, regional cerebral ^{123}I DEX binding was reduced in left temporal and right temporo-parietal regions in patients with mild probable AD compared with normal controls. Percentages of CSF space in ROIs were similar in patients and controls and had no influence on the results of this study. The new method was validated in an observer study showing equal ability to demonstrate differences between patients and controls in ^{123}I DEX binding across three observers. Further study is needed to determine whether muscarinic receptor binding with SPET can play a role in treatment selection for cholinomimetic therapy, in particular with newly developed selective muscarinic receptor agonists.

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