In Vivo Imaging of Muscarinic Cholinergic Receptors in Temporal Lobe Epilepsy with a New PET Tracer: [^{76}Br]4-Bromodexetimide

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Muscarinic acetyl cholinergic receptors (mAChRs) may be involved in the pathophysiology of partial epilepsy. Previous experimental and imaging studies have reported medial temporal abnormalities of mAChR in patients with medial temporal lobe epilepsy (MTLE). Suitable radiotracers for mAChR are required to evaluate these disturbances in vivo using PET. Dexetimide is a specific mAChR antagonist that has been labeled recently with ^{76}Br. This first study in humans focused on regional distribution and binding kinetics of [^{76}Br]4-bromodexetimide (BDEX) in patients with MTLE. **Methods:** Ten patients with well-lateralized MTLE had combined MRI, ^{18}F-fluorodeoxyglucose (FDG) PET and ^{76}Br-BDEX PET studies. Time-activity curves were generated in PET-defined regions of interest, including the medial, polar and lateral regions of the temporal lobe; the basal ganglia; the external and medial occipital cortex; and the white matter. **Results:** The highest radioactivity concentration was observed in the basal ganglia and in the cortical regions, whereas radioactivity was lower in the white matter. On late images of PET studies, ^{76}Br-BDEX uptake was statistically significantly decreased only in the medial temporal region ipsilateral to the seizure focus (1.37 ± 0.28, $P < 0.01$) as determined by FDG PET imaging, anatomic MRI and electroencephalogram correlation, compared with the contralateral medial temporal region (1.46 ± 0.31). **Conclusion:** ^{76}Br-BDEX concentration is reduced in the temporal lobe ipsilateral to the seizure focus in patients with MTLE. This preliminary study suggests that ^{76}Br-BDEX is a suitable radiotracer for studies of mAChR in humans. Further studies are required to investigate the potential value of ^{76}Br-BDEX PET in other neurological disorders with muscarinic disturbances.

Key Words: muscarinic receptors; bromodexetimide; PET; temporal lobe epilepsy

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The central nervous cholinergic systems may be involved in the generation and the propagation of epileptic discharges, because cholinergic agonists such as carbachol or pilocarpine are known to induce focal (*1*) or generalized (*2*) epilepsy in animals; whereas in other focal epilepsy models, acetylcholine may play a protective role (*3*). Furthermore, cholinergic fibers may be altered in focal epilepsy. Green et al. (*4*) using acetylcholinesterase found a loss of hippocampal cholinergic fibers in surgically resected temporal lobe specimens that may result from a general destruction or a retrograde transsynaptic degeneration of the afferent axons in the regions of pyramidal cell. Conversely, increased activity of choline acetyltransferase and acetylcholinesterase was found in the actively epileptic human cerebral cortex (*5*).

Some studies suggested that focal epilepsy might also be linked to muscarinic central cholinergic receptors. McNamara (*6*) reported a significant loss of muscarinic receptors in the amygdaloid region of kindled rats. This finding has been recently extended to human mesial temporal lobe epilepsy (MTLE) with SPECT. SPECT studies reported a decrease of in vivo binding of ^{123}I-iododexetimide (IDEX), a muscarinic receptor antagonist, in the epileptogenic temporal lobe of MTLE patients (*7–9*). This study was designed to confirm these results and to gain further information into muscarinic receptor abnormalities in temporal lobe epilepsy. We imaged and quantified muscarinic acetylcholine receptors using PET and [^{76}Br]4-bromodexetimide (BDEX), a recently developed muscarinic acetyl cholinergic receptor (mAChR) PET tracer, in patients with diagnosed unilateral MTLE.

MATERIALS AND METHODS

Patients

The study population included 10 patients (4 men, 6 women; mean age 36.6 ± 10.3 y) with refractory MTLE who were candidates for surgical treatment. All patients underwent similar presurgical evaluation as previously described (*10*), including medical, neurological and neuropsychological examinations, ictal and interictal video-electroencephalogram (EEG) monitoring (*11*), intracarotid amytal test, brain MRI (*12*) and fluorodeoxyglucose (FDG) PET examinations (*13*). The patients were selected on the following criteria: (a) clinical and EEG data congruent with a unilateral temporal lobe seizure onset, (b) unilateral hippocampal atrophy assessed by MRI volumetric measurements without any other underlying structural lesion and (c) unilateral temporal hypometabolism on FDG PET. Five patients had a left-sided hippocampal atrophy and 5 had a right-sided atrophy. All patients provided

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written informed consent in agreement with the Local Ethical Committee.

Radiopharmaceutical Preparation

The method used for the synthesis of bromodexetimide and ^{76}Br-BDEX (Fig. 1) was previously described (*14*). Briefly, the hydrochloride salt of dexetimide was neutralized using sodium carbonate in water, and the free base was extracted in ether. The benzyl group was then removed by catalytic hydrogenolysis with formic acid as the hydrogen donor. The norbenzyldexetimide was alkylated using 4-bromo-benzylbromide or 4-trimethylsilybenzyl-bromide. ^{76}Br-BDEX was prepared by electrophilic bromodesilylation using chloramine-T and no-carrier-added ^{76}Br that was produced by irradiation of arsenic with a beam of 30 MeV [^{3}He] ions provided by the cyclotron. Radiochemical and chemical purities assessed by radio thin-layer chromatography on silica gel plates and reverse phase high-performance liquid chromatography were 98%, and specific activity calculated at the end of synthesis was higher than 22 GBq/μmol (600 mCi/μmol). Pharmacological characterization of ^{76}Br-BDEX was recently reported in rats and baboons by Loc'h et al. (*15*). For injection in patients, ^{76}Br-BDEX was dissolved in a mixture of saline ethanol (98/2) and was filtered through a 0.2 μm sterile membrane (Millex-FG; Millipore SA, Molsheim, France).

PET Experimental Procedure

PET scans were performed on a dedicated high-resolution head PET camera (ECAT 953/31B Siemens; CTI, Knoxville, TN). This tomograph has 5.8-mm in-plane and 5-mm axial resolution (*16*). Thirty-one transverse sections of the brain spaced 3.37 mm apart were simultaneously acquired in the hippocampal plane (*17*), that is, the plane parallel to the long axis of the hippocampus according to a previously described method (*18*).

The investigations were made interictally under close clinical supervision. Ambient light and noise in the PET camera room were controlled in a standardized fashion (dim light and quiet environment, with patients' eyes open). After intravenous injection of a mean dose of 37 MBq (1 mCi) ^{76}Br-BDEX (specific activity ranged from 21.5 GBq [0.58 Ci] to 52.8 GBq [1.43 Ci]/μmol), brain radioactivity was measured in a consecutive series of time frames for up to 315 min. Two experimental procedures were performed. First, a 315-min dynamic study was performed in the first 5 patients to evaluate the tracer's kinetic. Beginning at the time of injection, the frame sequence consisted of two 5-min frames, two 10-min frames, one 15-min frame and one 20-min frame. Then three frames were registered as follows: a 20-min image acquisition beginning at 110 min after injection, a 30-min image acquisition beginning at 195 min after injection and a 30-min image acquisition beginning at 285 min after injection. The lapse in the frames allowed the patients to rest between the acquisitions. During the study, patients were carefully monitored for head movements and were immediately repositioned if necessary before and during the scan. Preliminary results of this dynamic study demonstrated that radioactivity reached a plateau 90 min after injection; therefore, a second simplified protocol was performed on the basis of these results. A 60-min image acquisition beginning 90 min after injection was performed in the last 5 patients. In all patients, reconstructed images were corrected for attenuation by use of ^{68}Ge transmission scans. Reconstruction was performed using the Hanning's filter with a frequency cutoff of 0.5 cycles/mm, the reconstructed resolution was 8.8 mm in the axial plane.

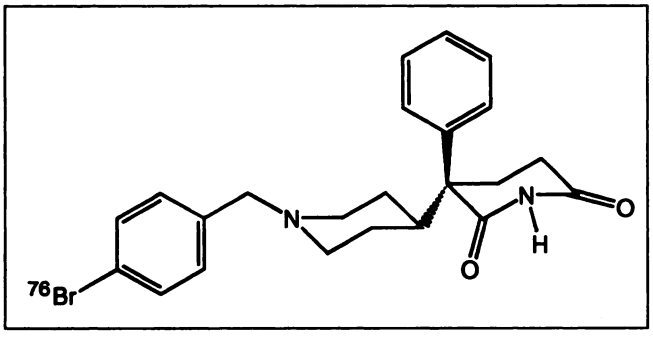

FIGURE 1. Chemical structure of [^{76}Br]4-bromodexetimide.

Regions of Interest

Regions of interest (ROIs) were directly delineated on the PET images. The exact anatomic delineation of these ROIs was concurrently visually checked on the MR images (Fig. 2). ROIs included medial and lateral temporal cortices defined on three contiguous images; medial and lateral occipital cortices defined on four contiguous images; basal ganglia (head of the caudate nucleus, thalamus); white matter adjacent to the insula; and temporal regions including the medial temporal cortex (i.e., the hippocampus and the adjacent parahippocampal gyrus), the temporal pole, the anterior part of the temporal neocortex, the middle part of the lateral temporal neocortex and the posterior part of the lateral temporal neocortex.

Data Analysis

Radioactivity concentration, measured in all these ROIs after correction for ^{76}Br decay, was expressed as percentage injected dose (%ID) per liter of tissue. Because muscarinic receptors are not present in the white matter, white matter activity was assumed to represent only nonspecific uptake (*19*). The regional specific uptake index was therefore calculated as the ratio of regional total concentration to nonspecific concentration. We first determined the dynamic characteristics of the tracer by studying the regional concentrations of ^{76}Br-BDEX uptake as a function of time. According to a previous study performed in our laboratory (*15*), which established the in vitro and in vivo properties of ^{76}Br-BDEX (affinity, stereospecificity, selectivity, saturation, displacement and localization of binding to mAChR), we postulated that the images acquired during the radioactivity plateau were receptor dependent. Thus, we decided to calculate ^{76}Br-BDEX binding for all patients at 120 min after injection at a time when the ^{76}Br-BDEX concentrations remain on plateau. We also calculated ^{76}Br-BDEX uptake in the earliest times in the 5 patients who underwent the dynamic study. We then calculated the specific-to-nonspecific uptake ratio for each region in the epileptogenic and contralateral hemispheres. ^{76}Br-BDEX uptake was then compared in each ipsilateral and contralateral region using a paired Student *t* test with correlated groups.

RESULTS

Figure 3 shows an example of ^{76}Br-BDEX binding and FDG uptake in a patient with left-sided hippocampal sclerosis. There is a decrease of ^{76}Br-BDEX concentration in the temporal lobe ipsilateral to the seizure focus. Yet, this abnormality is only restricted to the left medial temporal

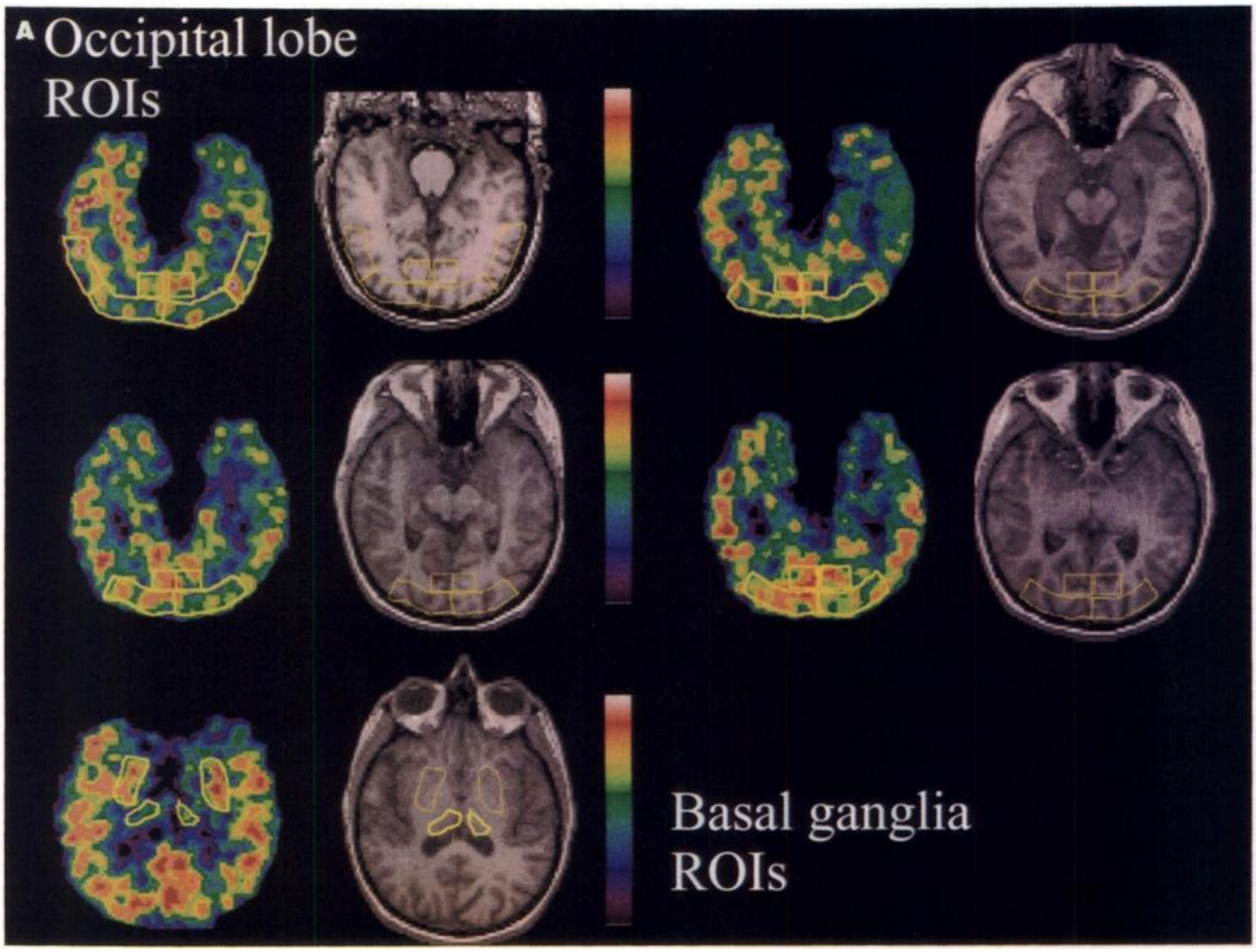

FIGURE 2. PET representation of regions of interest (ROIs) with MRI correspondence. (A) Occipital lobe and basal ganglia ROIs.

region as opposed to the much more widespread temporal lobe hypometabolism evidenced on the FDG images.

Time-dependent regional concentrations of ^{76}Br-BDEX evidenced a ^{76}Br-BDEX uptake higher in the basal ganglia and the cerebral cortex than in the rest of the brain. In all cerebral structures, maximal radioactivity was reached within 50 min, followed by a plateau (Fig. 4). A slow decrease of radioactivity was noted around 200 min after injection. In the white matter, the radioactivity also peaked at T0 + 50 min with a maximal level lower than in the rest of the brain and then decreased slowly until the end of the experiment. These results led us to assume that equilibrium between plasma and receptor-bound ^{76}Br-BDEX was obtained at the time of the plateau. At this time, time-dependent concentrations of ^{76}Br-BDEX were on a different level among the ipsilateral and contralateral medial temporal regions for all the individual patients (Fig. 5).

Accordingly, a statistically significant decrease of ^{76}Br-BDEX binding was found only in the medial epileptogenic temporal region on the late images using both radioactivity concentrations ($P < 0.005$) (Table 1) and total binding-to-nonspecific uptake ratios ($P < 0.01$). ^{76}Br-BDEX specific binding tended to be lower in the temporal pole region and in the anterior part of the temporal neocortical region on the side of the focus compared with contralateral homologous regions, but this difference was not significant ($P = 0.19$ and 0.29, respectively). Statistical analysis also failed to find a significant difference between paired regions in the ipsilateral and contralateral regions of the middle and posterior temporal neocortex and the occipital lobe, as well as in the basal ganglia.

In addition, in the earliest times, there was no significant decrease of ^{76}Br-BDEX binding in the medial temporal region (Fig. 6) or other regions.

DISCUSSION

In Vivo Distribution of [^{76}Br]4-Bromodexetimide

We report on a PET study using ^{76}Br-BDEX in humans. Five distinct mAChR subtype genes (m1–m5) have been cloned in humans (*20*), whereas four subtypes mAChR (M1–M4) have been characterized in studies of functional and radioligand binding (*21*). The M1 receptors that are the main type found in the human brain dominate in the cerebral cortex, caudate nucleus, hippocampus, nucleus accumbens

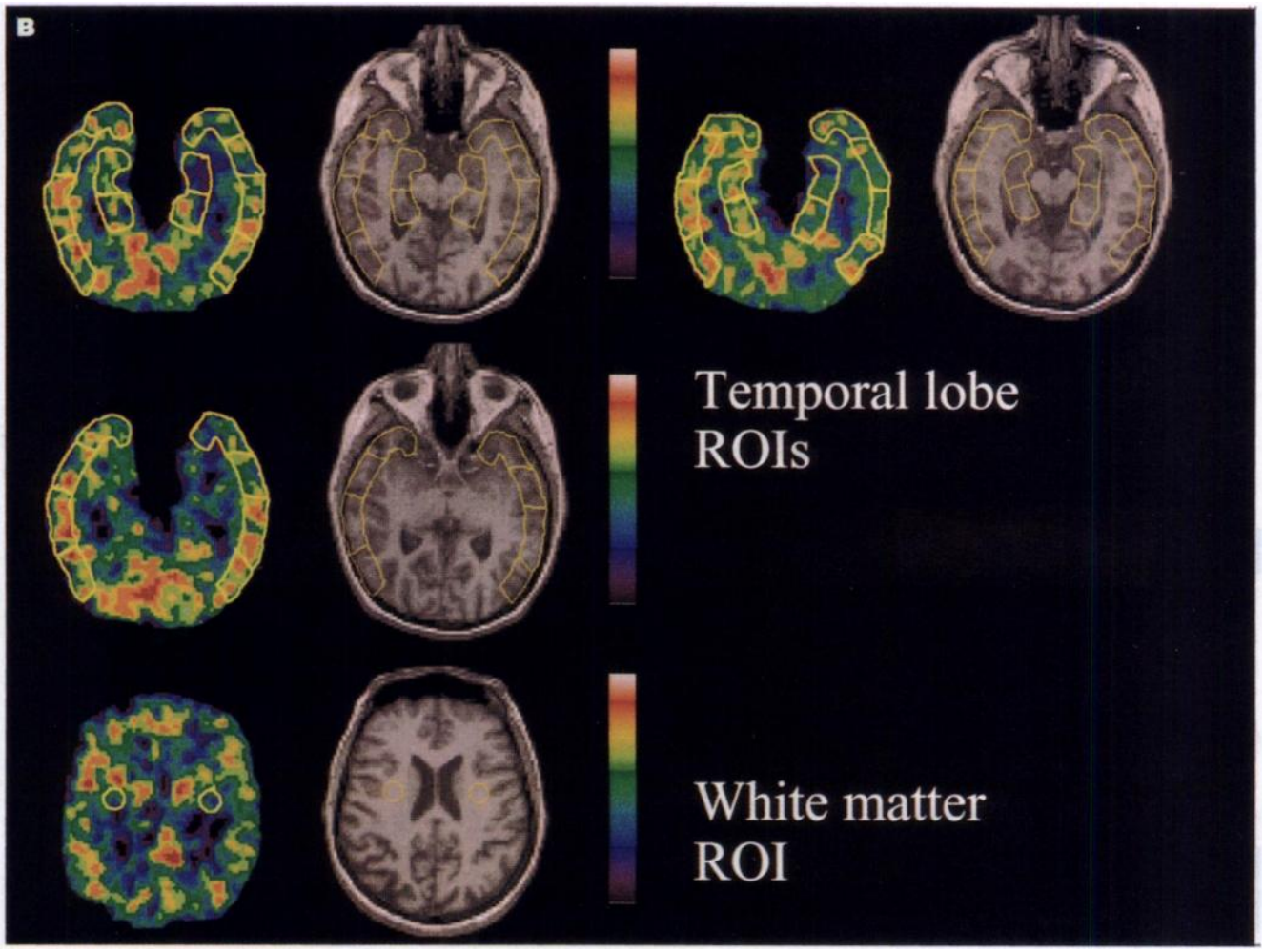

FIGURE 2. (Continued.) (B) Temporal lobe and white matter ROIs.

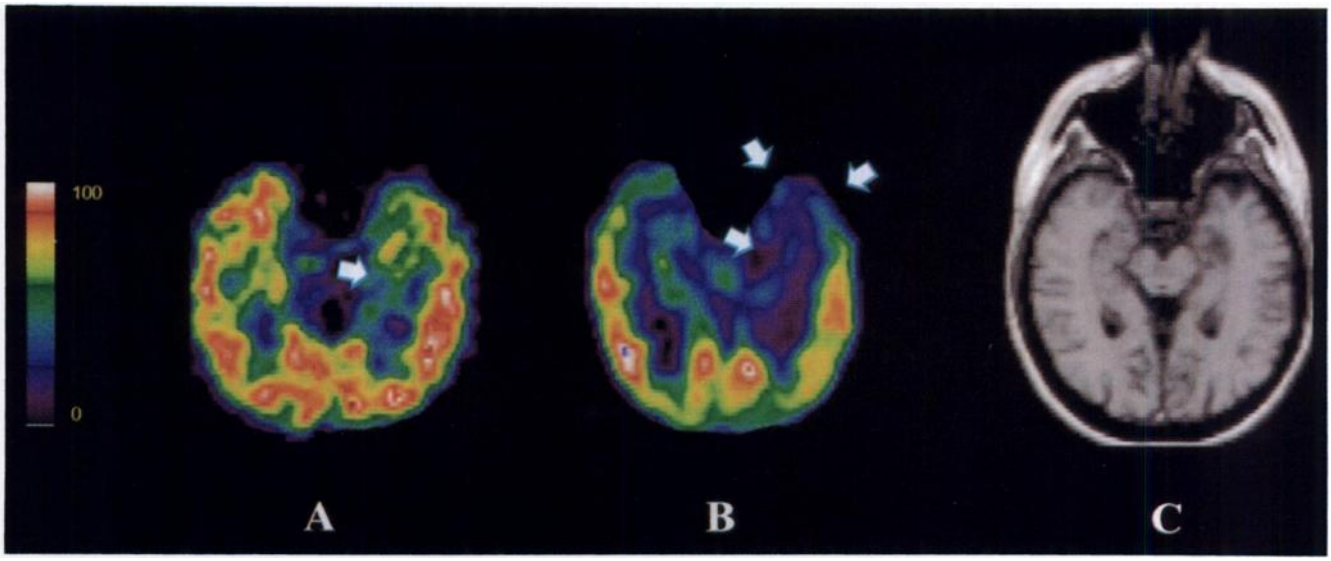

FIGURE 3. Axial PET images show hippocampal decrease of ^{76}Br-BDEX binding (A), large anteromedial temporal decrease of FDG uptake (B) and corresponding MR image (C) (T1-weighted sequence) in patient with left-sided hippocampal sclerosis.

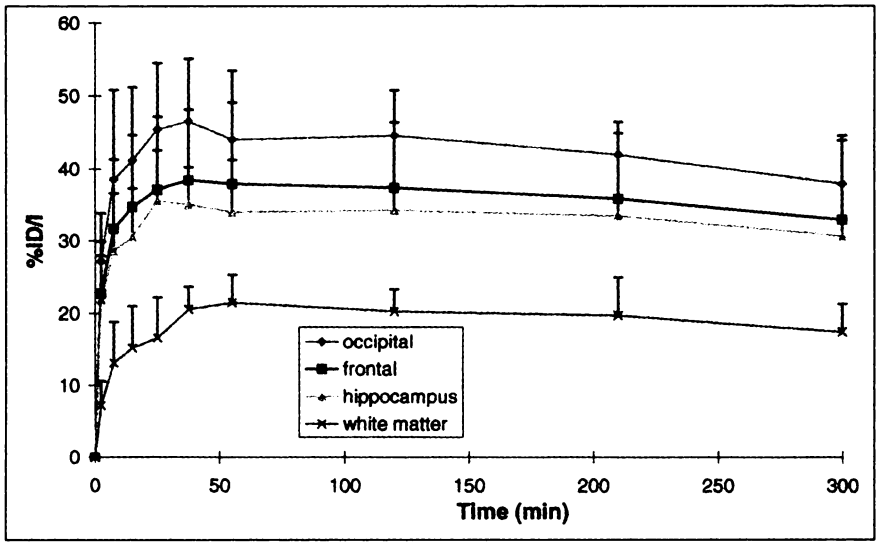

FIGURE 4. PET dynamic study. Time-course concentrations of ^{76}Br-BDEX in brain regions contralateral to seizure focus in 5 MTLE patients (mean ± SD). Maximal uptake was observed in cerebral cortex with lowest uptake in white matter. %ID/L = percentage injected dose per liter.

and globus pallidus. The M2 type dominates in the thalamus, brain stem, and cerebellum (*22,23*), the M3 type dominates in the cerebral cortex and hippocampus and the M4 type is most abundant in the striatum (*24*). Because previous experimental in vitro studies (*25*) have demonstrated that ^{76}Br-BDEX binds to all four mAChR subtypes, we expected the in vivo distribution of ^{76}Br-BDEX to be consistent with the in vitro distribution of the muscarinic receptors. Because no brain abnormalities have been reported on the contralateral side to the epileptogenic focus in MTLE patients, we decided to investigate the regional distribution of ^{76}Br-BDEX in the hemisphere contralateral to the epileptogenic focus. We confirmed that the human in vivo regional distribution of ^{76}Br-BDEX was consistent with the known in vitro cerebral distribution of mAChR in rats (*19,26*) and humans (*22*). We showed that cerebral ^{76}Br-BDEX uptake was higher in the striatum and in the cerebral cortex contralateral to the epileptogenic side and was lower in the white matter.

Muscarinic Receptor Binding Abnormalities in MTLE

The major finding of this study is that ^{76}Br-BDEX uptake is decreased in the medial temporal cortex on the side of the epileptic focus. Regionally decreased hippocampal mAChR uptake can be attributed to several mechanisms.

First, as recently suggested by Weckesser et al. (*9*), this reduction may be related to a partial volume effect due to hippocampal atrophy. Nevertheless, the influence of atrophy on receptor imaging remains controversial. Weckesser et al. (*9*) recently estimated the influence of hippocampal atrophy on IDEX, the analog of ^{76}Br-BDEX for SPECT. Their results suggested that the reduction observed earlier in hippocampal ^{123}I-IDEX binding in MTLE patients was due to a decrease in hippocampal volume rather than to a decrease in receptor concentration. In this SPECT study, the method used to correct the partial volume was technically different from the physiological situation. Furthermore, in an animal kindling model of partial epilepsy, McNamara (*6*) demonstrated selective reductions in the number of muscarinic cholinergic

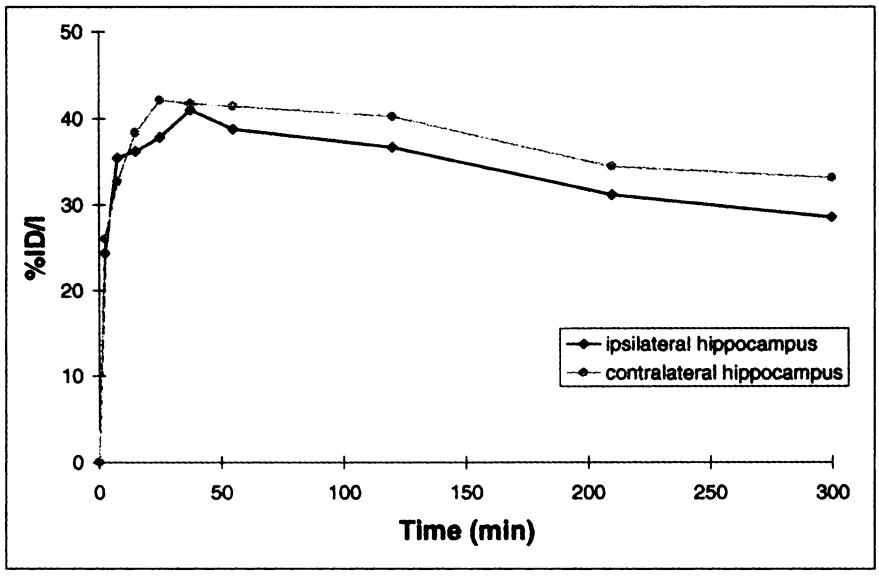

FIGURE 5. Time concentrations of ^{76}Br-BDEX in ipsilateral and contralateral medial temporal regions in 1 MTLE patient. %ID/L = percentage injected dose per liter.

TABLE 1
Regional Distribution of Radioactivity: Absolute Values to Radioactive Percentage Injected Dose per Liter of Tissue at Time + 120 Minutes in 10 MTLE Patients

Region	Ipsilateral (mean ± SD)	Contralateral (mean ± SD)
Medial temporal	31.7 ± 5.1*	33.9 ± 6.0
Temporal pole	33.1 ± 8.1	35.9 ± 8.7
Anterior temporal neocortex	35.9 ± 6.6	38.6 ± 10
Middle temporal neocortex	40.1 ± 9.0	40 ± 10.4
Posterior temporal neocortex	40.2 ± 8.4	39.8 ± 9.8
External occipital cortex	39.0 ± 9.0	39.2 ± 10.0
Medial occipital cortex	44.1 ± 8.3	43.9 ± 8.3
Basal ganglia	49.6 ± 13.2	49.3 ± 12.5
White matter	26.2 ± 2.5	26.4 ± 5.9

*$P < 0.005$.
MTLE = medial temporal lobe epilepsy.

receptor binding sites in both amygdaloid regions of rats killed after completion of kindling. Boundy et al. (*8*) have also demonstrated in a comparative SPECT study using both ^{123}I-IDEX and hexamethyl propyleneamine oxime (HM-PAO) tracers in MTLE patients that ^{123}I-IDEX binding was reduced in the hippocampal region ipsilateral to the seizure focus and that this decrease was significantly greater than the blood flow asymmetry. In addition, as suggested by Weckesser et al. (*9*), we evaluated early and late ^{76}Br-BDEX binding, assuming that early ^{76}Br-BDEX accumulation was mainly governed by unspecific effects like cerebral blood flow, whereas late distribution reflected receptor density. We clearly demonstrated that the reduction in ^{76}Br-BDEX binding was only statistically significant on the late images. It may thus be hypothesized that this increasing asymmetry over time reflects a receptor-associated defect.

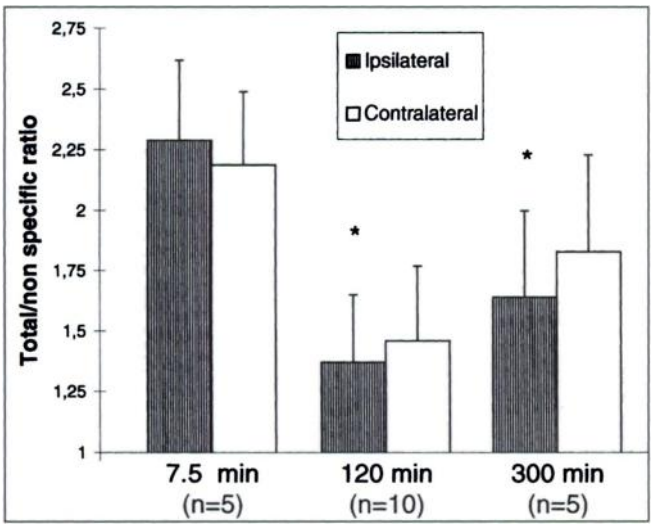

FIGURE 6. Ipsilateral and contralateral to focus ^{76}Br-BDEX concentrations in medial temporal region at 7.5, 120 and 300 min.

Second, ^{76}Br-BDEX decrease can be attributed to neuronal loss observed in hippocampal sclerosis. Burdette et al. (*27*) have recently demonstrated that decreases in central benzodiazepine binding reflect regional decreases in neuronal cell counts rather than significant changes in binding site affinity. Furthermore, we noted a tendency to a decrease, although not significant, in ^{76}Br-BDEX binding in the temporal pole and the anterolateral temporal neocortex ipsilateral to the seizure focus, regions that are not affected by the neuronal loss. These results are in agreement with the decrease of FDG uptake observed in this study (Fig. 3) and in previous FDG PET studies. Numerous FDG PET studies have effectively demonstrated a significant decrease in FDG uptake in the temporal cortex ipsilateral to the epileptic focus with an hypometabolic region usually considerably larger than the structural abnormalities or the EEG focus (*18,28,29*), involving both the temporal pole and the anterior part of the temporal neocortex. The mechanism of this widespread hypometabolism in temporal lobe epilepsy remains unknown but our results suggest that it might be related to a cholinergic disturbance.

An alternative hypothesis that explains the significant decrease of ^{76}Br-BDEX binding in the medial temporal region ipsilateral to the seizure focus is the existence of a specific mAChR decrease. A study that addressed results of chronic enhanced cholinergic transmission in rats demonstrated a decrease in the mAChR number by downregulation (*30*). Such a mechanism may be hypothesized in the epileptic human brain, because some authors have found an increase of endogenous acetylcholine in the actively epileptic human cerebral cortex (*5*). Another hypothesis for the significant decrease of ^{76}Br-BDEX binding is increased endogenous acetylcholine competing with cholinergic tracers (i.e., ^{76}Br-BDEX) in binding to mAChR as suggested by Müller-Gärtner et al. (*7*), but no experimental study further supports this hypothesis.

CONCLUSION

^{76}Br-BDEX binding is decreased in the medial temporal region on the side of the epileptic focus, suggesting a cholinergic disturbance in MTLE. However, we cannot state whether this decrease is related to a primary cholinergic dysfunction or is the consequence of the neuronal loss. Other studies are needed to investigate this issue in patients with MTLE and without hippocampal sclerosis and in patients with other neurological diseases. In vitro studies of hippocampal resected tissues of MTLE patients undergoing surgery may also provide further information.

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