Neuronal nAChR stereoselectivity to non-natural epibatidine derivatives

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Abstract The frog toxin epibatidine is one of the most powerful ligands of the neuronal nicotinic receptors and derivatives show promising possibilities for labeling in positron emission tomography studies. In an attempt to reduce epibatidine toxicity, new methyl derivatives were synthesized, tested in positron emission tomography imaging and in electrophysiology. labeling as well as physiological experiments highlighted the differences in sensitivity of the neuronal nicotinic acetylcholine receptors between two methyl enantiomers and the reduction in sensitivity caused by introducing the methyl group. At present, epibatidine derivatives seem the most promising compounds for in vivo labeling of neuronal nicotinic acetylcholine receptors.

Key words: Nicotinic acetylcholine receptor; Brain; Epibatidine; Pharmacophore; Imaging

1. Introduction

With the introduction of tobacco plants on the second voyage of Christopher Columbus its use and smoking addiction quickly spread amongst a large fraction of the population. Although it was recognized earlier that the alkaloid nicotine is the active compound in tobacco leaves and that it is a potent agonist of the cholinergic system at the neuromuscular and ganglionic synapses, the mechanisms by which it causes addiction remained obscure [1,2]. The identification of an entire family of genes encoding the neuronal nicotinic acetylcholine receptors (nAChRs) that are specifically expressed at the neuromuscular junction or in nerve cells shone a new light on the mechanisms by which nicotine can exert its action in the central nervous system [3,4]. One of the critical findings was the observation that neuronal nAChRs display a wide pattern of physiological and pharmacological profiles and that a given set of subunits is expressed in a well-defined brain area [5–7]. For instance, it was shown that the major brain neuronal nAChR results from the assembly of the α4 and β2 subunits. Moreover, a high level of expression of this class of receptor was observed on dopaminergic neurons that are known to participate in reward and addictive behaviors [8,9].

With the introduction of in situ hybridization using specific mRNA probes it has become possible to map the pattern of expression of the identified neuronal nAChR subunits in rat, mouse or human brains [7,10]. Although allowing a high spatial resolution the use of this technique cannot be employed for in vivo measurements. The development of new tools is therefore required to establish the relationship between the in situ pattern of neuronal nAChR expression in a defined brain area and physiological behavior. The availability of positron emission tomography (PET) ligands would constitute a first alternative to postmortem hybridization or biochemical studies and make it possible to correlate nicotine labeling with cognitive or behavioral status.

Initial studies performed with radioactive nicotine have revealed the inadequacy of this compound in PET studies and that more specific ligands must be identified to obtain proper spatial and temporal resolutions [11,12]. The identification of a new frog toxin, epibatidine, which displays a very high affinity for the neuronal nAChRs, opened new possibilities to design synthetic compounds of potential interest for PET monitoring [13–17]. We produced *N*-methylated epibatidine for this purpose [13]. Recently this and other methylated epibatidine analogs were synthesized, with very similar biological properties (e.g. receptor affinity and toxicity) [16]. The aim of this work is to examine the properties of the optical isomers of *N*-methylepibatidine on reconstituted neuronal nAChRs and to determine their potential application for PET investigations.

2. Materials and methods

2.1. Chemistry

All chemicals were purchased in analytical quality from Aldrich, Fluka, Merck or Sigma unless otherwise mentioned. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Gemini 200 and Gemini 300 spectrometers. Mass spectra were recorded on a Trio 2000 spectrometer (VG Organic, UK) using the positive ion mode with electrospray (ES+).

The separation of the enantiomers was performed by HPLC on a semi-preparative ChirobioticT column (250×10 mm) from Astec and Merck LaChrom equipment (D-7000, L-7100, L-7250, L-7450). Methanol was used in preparative HPLC quality from Merck. Optical rotations were measured with a Perkin Elmer 241 polarimeter at 23°C using a sodium lamp (D-line) and are reported as specific rotation [α] in degrees. The purity is reported in percent of enantiomeric excess (%ee). The ^{11}C-labeled compounds were produced as described in detail elsewhere [18] by methylation of the desmethyl precursor with [^{11}C]MeI.

2.2. PET studies in the rat

Male Sprague-Dawley rats (300–400 g) were anesthetized with Domitor and Hypnorm (0.25 ml/kg body weight), according to the procedure required by the veterinary department. Anesthesia was maintained during the study by subcutaneous injection of Hypnorm/Domitor (0.12 ml/kg body weight). A catheter was introduced into the tail vein. The rat was placed in the PET scanner (GE-Advance) with the whole body of the rat in the field of view. Prior to the PET

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Abbreviations: ACh, acetylcholine; nAChR, nicotinic receptor; CNS central nervous system; PNS, peripheral nervous system

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scans a transmission scan was performed. An amount of 1–2 MBq of the ^{11}C compound was injected. Data acquisition was started and 22 frames were collected 10×0.5 min, 5×1 min, 2×5 min, 4×10 min and 1×20 min.

Regions of interest (ROI) were drawn directly on the 'transaxial' PET scans, which are sagittal as a result of the positioning of the rat in the field of view. The activity in the ROIs was calculated from the absolute count rate by multiplication with area and slice thickness (0.425 cm). The result is displayed as percent injected dose. The amount of activity injected into the rat was calculated from a whole body ROI and the percentage of ^{11}C tracer in the brain was determined as a function of midframe time. The result of the whole body ROI was compared to the amount of activity injected into the rat determined by measuring the syringe before and after injection in a dose calibrator. The values determined by whole body ROI were in agreement with the ^{11}C dose determined with the dose calibrator.

2.3. Electrophysiology

Xenopus oocytes were prepared according to the standard procedure [19] and nuclear-injected with 10 nl of buffer containing equal concentrations (0.1 μg/μl) of α and β subunit cDNAs or 0.2 μg/μl of α7. Recordings were made using the two-electrode voltage clamp technique. During the experiments, oocytes were continuously superfused with control solution containing: 82.5 mM NaCl, 2.5 mM KCl, 5 mM HEPES, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4 adjusted with NaOH. All chemicals were obtained from either Fluka or Sigma (Buchs, Switzerland).

Dose-response relationships were adjusted to the empirical Hill equation

$$y = \frac{1}{1 + \left(\frac{EC_{50}}{x}\right)^{n_H}} \quad (1)$$

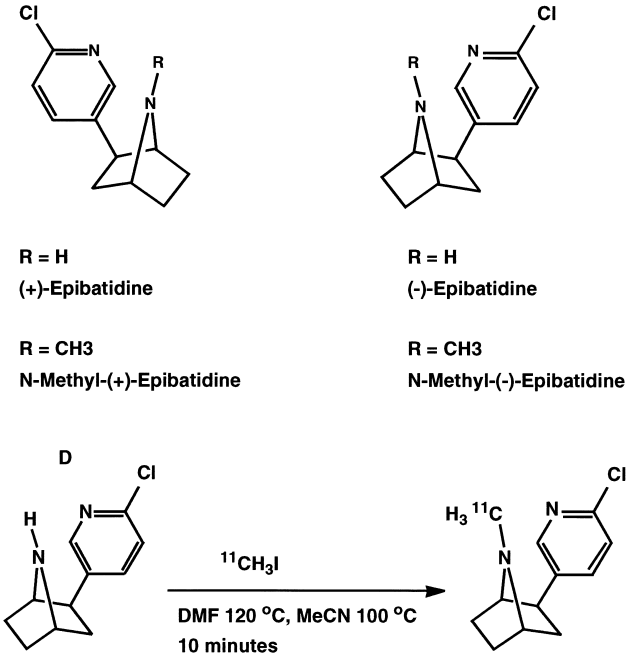

Fig. 1. Chemical structure of epibatidine and its methyl derivatives. The upper panel presents the two enantiomers of epibatidine or its methyl derivative. Chemical synthesis of the radiolabeled compound is schematized in the lower panel.

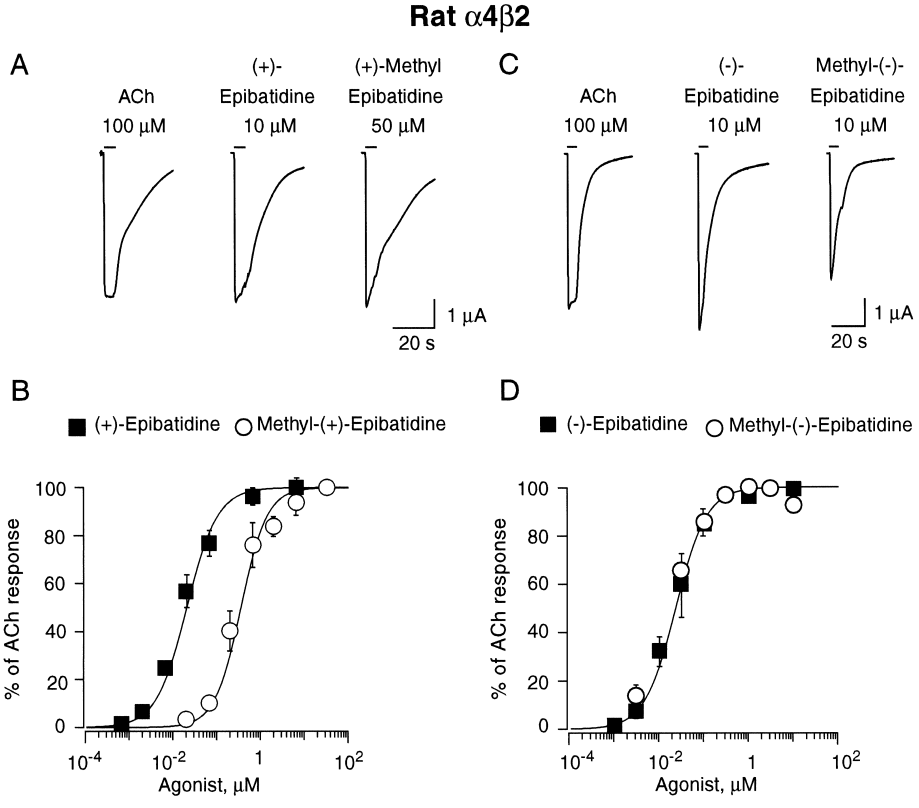

Fig. 2. Currents evoked by epibatidine and its derivatives on the major brain α4β2 nAChR. A: Typical currents evoked in a responsive oocyte by short application (2 s) of ACh, (+)-epibatidine and methyl-(+)-epibatidine at saturating concentration. B: Dose-response relationships for (+)-epibatidine and methyl-(+)-epibatidine (mean of four cells). Lines through the data points correspond to the best fits obtained with Eq. 1. C: Currents evoked, in another cell, as in A for ACh, (−)-epibatidine and methyl-(−)-epibatidine. D: Dose-response relationships for (−)-epibatidine and methyl-(−)-epibatidine (mean of four cells, values in Table 1). Data in B and D were normalized with respect to currents evoked by a saturating ACh concentration.

where y = the fraction of activated current, EC_{50} = concentration of agonist evoking a current of half maximal amplitude, n_H = Hill coefficient, x = agonist concentration.

Dose-response curves for (+)- and (−)-epibatidine and their analogs were normalized on an acetylcholine (ACh) saturating concentration. All values indicated throughout the text are given with their respective S.E.M. Cells were held throughout the experiments at −100 mV.

2.4. Toxicology

The acute intravenous toxicity of methylepibatidine was estimated in mice and rats by a specialized outside agency (RCC Research and Consulting Co. Ltd., Itingen, Switzerland). A racemic mixture of methylepibatidine was intravenously administered to groups of five male and five female animals. The doses were 0.04, 0.4, 8 and 80 μg/kg for the HanIbm:WIST (SPF) rats and 0.05, 0.5, 10 and 100 μg/kg for the HanIbm:NMRI (SPF) mice. Surviving animals were examined four to five times on day 1 and once daily on days 2–15 for clinical signs. Mortality/viability were recorded together with clinical signs at the same time intervals. All animals were necropsied and examined macroscopically.

3. Results and discussion

Neuronal nAChRs are integral membrane proteins that are thought to result from the assembly of five subunits around an axis of pseudosymmetry [20]. Earlier physiological and pharmacological experiments have revealed that ganglionic receptors from the peripheral nervous system (PNS) display patterns of agonists and antagonists clearly distinguishable from those of the muscle receptor [2,20]. Reinforced by many recent studies, these differences can be attributed to a particular expression of neuronal subtypes (reviewed in [20]). Furthermore, it was shown that ganglionic receptors contain at least the α3 and β4 subunits while central nervous system (CNS) receptors are thought to result from the assembly of α4 with β2 [7,21]. A less clear distinction can be made when considering receptors containing the α7 subunit which have been shown to be present in both the CNS and the PNS [6,22–24]. To examine the effects of epibatidine and its *N*-methyl derivatives on the CNS and PNS nAChR types we determined the action of these compounds on neuronal receptors reconstituted in *Xenopus* oocytes.

In a recent contribution Horti et al. [16] studied *N*-alkylated epibatidine derivatives with various halogens, fluorine, bromine and iodine, at the position of the chlorine on the pyridine ring. They found affinities to rat brain homogenates, mouse brain distributions and toxicity towards mice which were all very similar. We concentrated on the differences between the enantiomeric *N*-methyl compounds in their dynamic behavior in the rat brain and in their activation of various nAChR subtypes.

A schematic representation of the two enantiomers of epibatidine and *N*-methylepibatidine employed throughout this work is given in Fig. 1.

3.1. Sensitivity of the rat α4β2 to epibatidine and methylepibatidine

Oocytes were injected with rat cDNA combinations α4β2 and their physiological properties were examined using a dual-

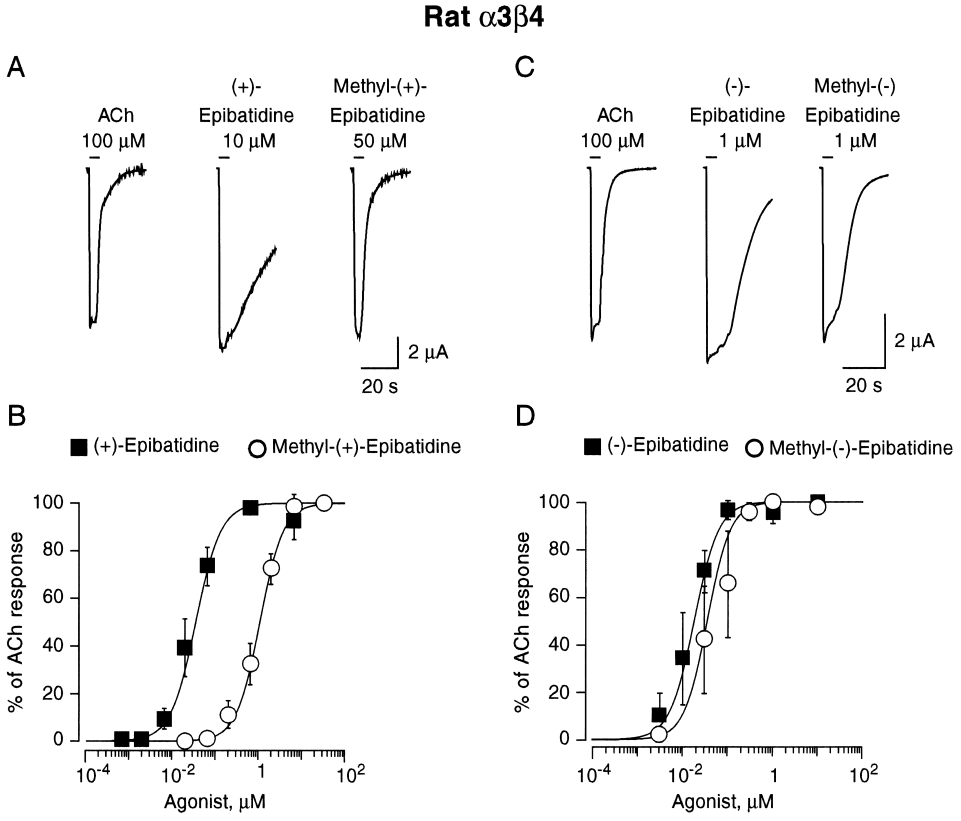

Fig. 3. Currents evoked by epibatidine and its derivatives on the ganglionic α3β4 nAChR. A and C: Typical currents evoked in a responsive oocyte by a short application (2 s) of ACh, epibatidine and its derivative at saturating concentration as in Fig. 2A,C. B: Dose-response relationships for (+)-epibatidine and methyl-(+)-epibatidine (mean of four cells). Lines through the data points correspond to the best fits obtained with Eq. 1. D: Dose-response relationships for (−)-epibatidine and methyl-(−)-epibatidine (mean of four cells, values are indicated in Table 1). All procedures were as described in Fig. 2.

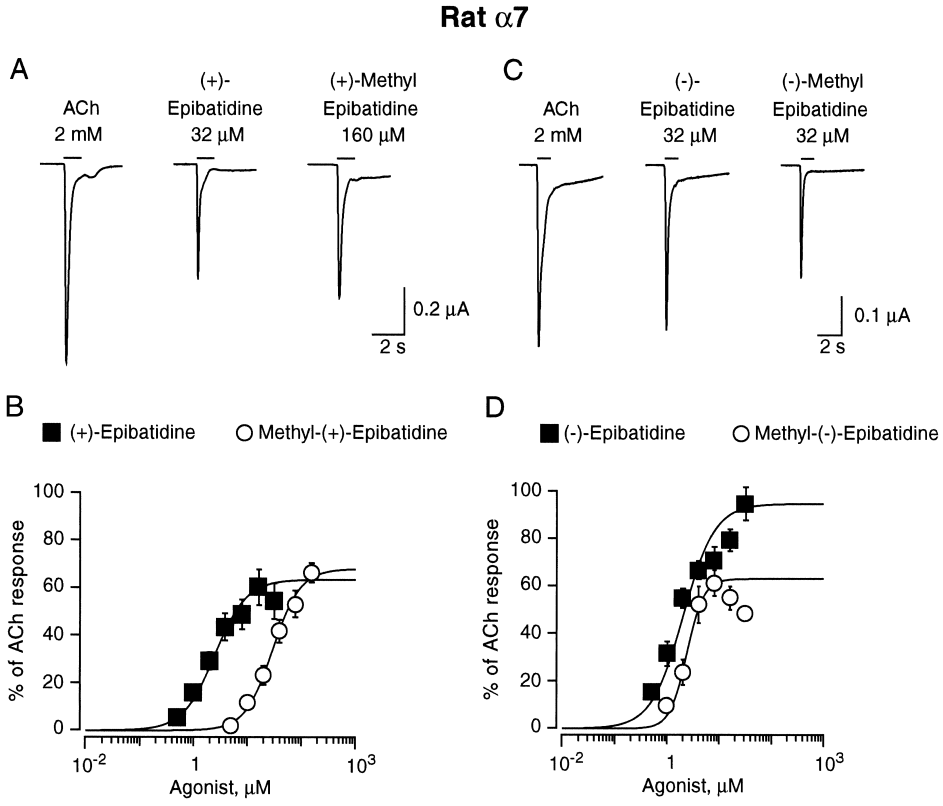

Fig. 4. Sensitivity of the α7 nAChR to epibatidine and its methyl derivatives. Traces recorded in response to (+) enantiomers are shown in A while currents evoked by the (−) enantiomers are shown in C. Note the differences in amplitude of the currents evoked by (+)- and (−)-epibatidine. B and D: Ddose-response relationships of the homomeric α7 nAChR (mean of four cells, see Table 1) to epibatidine. Data were obtained and processed as in Fig. 2.

electrode voltage clamp. Typical currents evoked, in a responsive oocyte, by acetylcholine (ACh), (+)-epibatidine and methyl-(+)-epibatidine are illustrated in Fig. 2A. The equal amplitude of currents evoked by these saturating test pulses indicates that these three compounds act as full agonists on the CNS receptor. Determination of dose-response curves (Fig. 2B) over a broad range of agonist concentrations revealed that the α4β2 receptor is about 15-fold more sensitive to epibatidine than its equivalent methyl derivative. When the same experiments were repeated with the (−) enantiomers a different picture was observed. Although no differences in amplitude could be observed between the currents evoked by ACh and (−)-epibatidine a small reduction was observed with methyl-(−)-epibatidine (Fig. 2C). On average, however, no significant differences could be detected between these three agonists. The small reduction observed on these currents can be partly attributed to the fastest desensitization of the receptor to methyl-(−)-epibatidine. Surprisingly, however, dose-response relationships (Fig. 2D) yielded no differences in apparent affinity between (−)-epibatidine and methyl-(−)-epibatidine indicating that introduction of the methyl side chain does not alter the receptor sensitivity for the (−) enantiomer (data summarized in Table 1).

3.2. Sensitivity of the ganglionic α3β4 epibatidine and its derivatives

Analysis of the currents evoked by the two enantiomers of epibatidine and methylepibatidine were carried out as described above for the CNS nAChR subtype. As shown in Fig. 3A, the (+) enantiomers of epibatidine and methylepibatidine both evoked robust currents of comparable amplitude to the natural agonist ACh. Moreover, as seen for the α4β2 nAChR, the α3β4 receptor displays a sensitivity roughly 30 times lower for the (+)-methyl compound than for the native toxin (Fig. 3B). Assessing the properties of the (−) enantiomers yielded results comparable to those observed with the α4β2 nAChR. Namely, while both epibatidine and methyl-epibatidine evoked currents of similar amplitude as those recorded in response to ACh (Fig. 3C), exposure to (−)-epibatidine and methyl-(−)-epibatidine yielded approximately similar half activation values (Fig. 3D). Thus, whereas this class of receptor does not distinguish between the (+)- and (−)-epibatidine enantiomers it is about 31-fold more sensitive to the (−) form of the methyl derivative than to its (+) enantiomer (see Table 1).

3.3. Sensitivity of the homomeric α7 nAChR

It is well documented that the homomeric α7 receptor is strikingly less sensitive to epibatidine than the heteromeric receptors [25]. For instance, whilst the major brain receptor displays a more than two orders of magnitude higher affinity for epibatidine than for ACh, the α7 sensitivity remains in the micromolar range. In addition, it is also known that this receptor shows a higher desensitization rate when exposed to epibatidine than when challenged with ACh. Current traces recorded in response to a fast application pulse display the typical profile reported earlier for the homomeric α7 receptor [23,26,27]. Unlike the heteromeric α4β2 or α3β4 receptors,

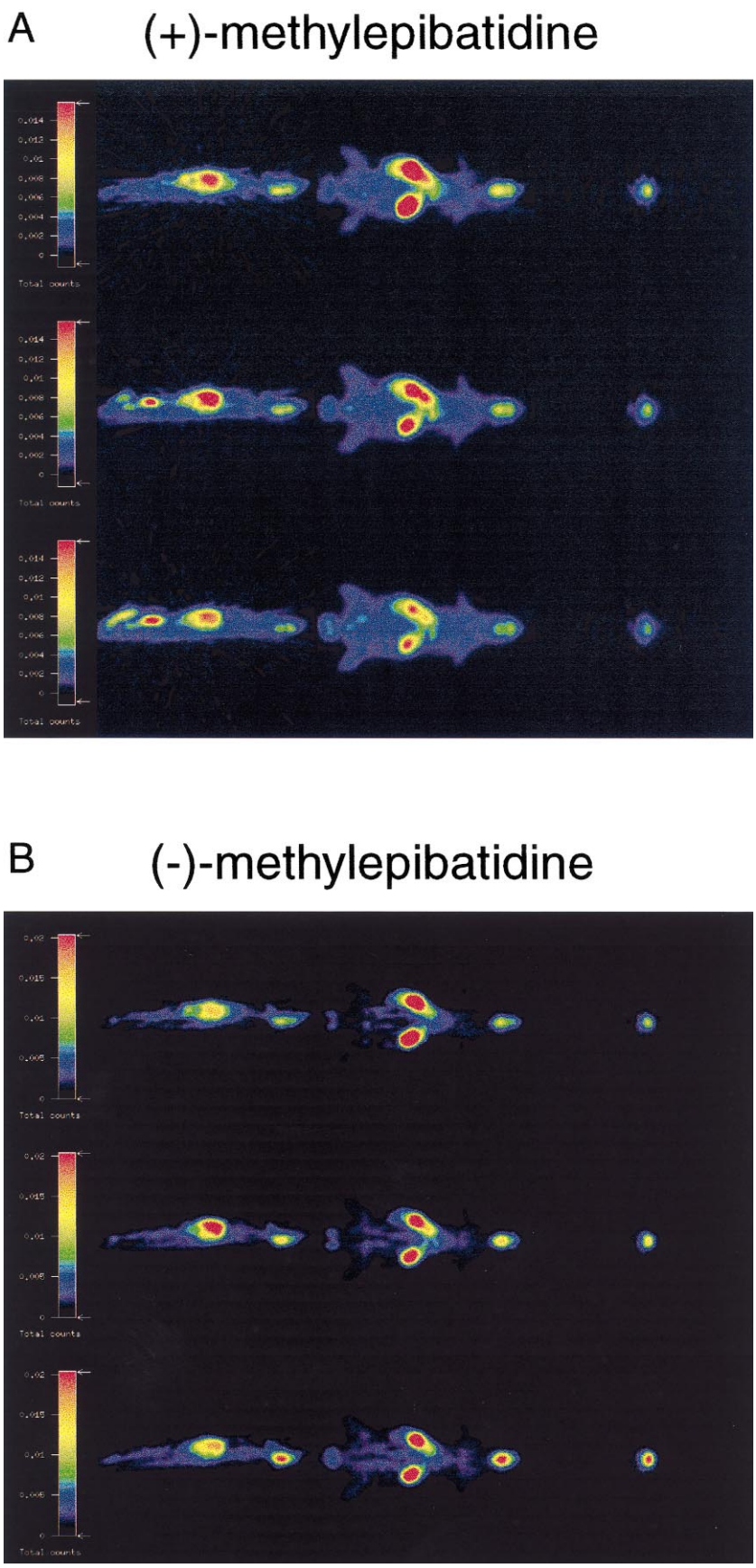

Fig. 5. PET images of [^{11}C]*N*-methyl-(+)- and (−)-epibatidine in rats. The region of the liver, urinary bladder and brain showed a high uptake of the tracer. The (+) isomer (A) showed a higher uptake in the region of liver, stomach and spleen compared to the (−) isomer (B). Because of the limited resolution of the PET images, only the total the brain activity curve is shown as % injected dose versus time in Fig. 6.

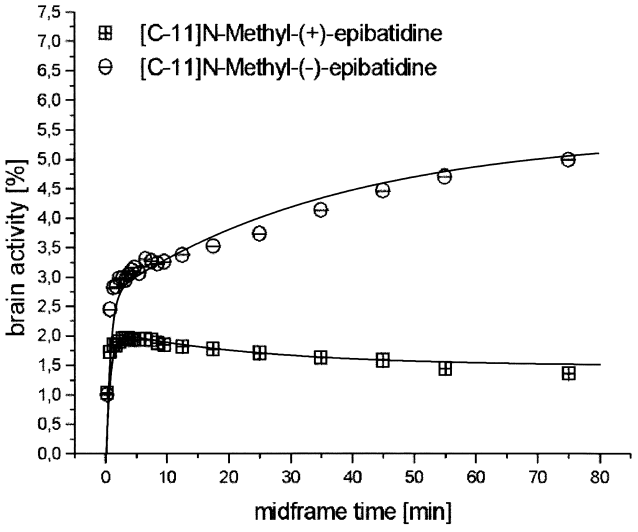

Fig. 6. Time course of [^{11}C]*N*-methyl-(+)- and (−)-epibatidine binding. Quantification of PET measurements was done as described in Section 2. Squares indicate the values measured with [^{11}C]*N*-methyl-(+)-epibatidine while data obtained with [^{11}C]*N*-methyl-(−)-epibatidine are represented by circles. Lines through data points were drawn to guide the eye.

however, both (+)-epibatidine and methyl-(+)-epibatidine evoked only a fraction of the currents caused by ACh exposure (Fig. 4A). Furthermore, as illustrated in Fig. 4B, adjunction of the methyl residue further decreased the apparent affinity of the α7 receptor to epibatidine by more than one order of magnitude. Data obtained with the (−) enantiomers are roughly comparable to the observation made on the heteromeric receptors (Fig. 4C). A marked difference is, however, found in the partial agonist mode of action of the (−)-methyl compound (see Fig. 4D and Table 1), which is not observed with the other receptor subtypes.

Taken together these data highlight the finding that introduction of a methyl side chain causes a decrease in the efficacy of the (+) enantiomer and introduces a stereoselectivity that is not observed with the native epibatidine. It is of interest to recall that although the neuronal nAChRs display a poor selectivity in the separation of enantiomers a difference between (−)- and (+)-nicotine has been widely documented. However, another chemical modification of epibatidine, the removal of the chlorine atom, introduced no stereoselectivity in the molecular recognition by the receptors [28]. Thus, the stereoselectivity caused by the introduction of a methyl substitute onto the nitrogen bridge of the bicyclocyclohexyl ring indicates that this segment of the molecule must be in closer interaction with the ACh binding site than the chlorine atom.

3.4. PET studies with N-[^{11}C]methyl(+)- and (−)-epibatidine

To characterize further the possibility of using these epibatidine derivatives in PET studies, a first set of experiments was conducted on rats. Pictures obtained after injection with *N*-[^{11}C]methyl(+)-epibatidine reveal that the concentration of this compound transiently increased in the brain and then progressively diminished (Fig. 5A). In contrast, when the same experiment was repeated with the (−) enantiomer a different pattern was revealed. While the signal observed in the liver or kidneys progressively decreased the brain uptake continued to increase (Fig. 5B). Comparison of the PET images and the brain uptake curves showed interesting differences in receptor binding properties of the methylated epibatidine stereoisomers. [^{11}C]*N*-methyl-(−)-epibatidine binds predominantly to one region in the rat brain. In contrast, two binding sites were seen for [^{11}C]*N*-methyl-(+)-epibatidine. The additional binding site seen very clearly for the (+) isomer was most likely the eyes of the rat, but because of limited resolution of the PET scan some other brain region such as olfactory bulb or frontal cortex may also show an enrichment of the tracer. Considerable differences were seen when comparing the time course of brain uptake for the enantiomers of [^{11}C]N-methyl-epibatidine. While [^{11}C]*N*-methyl-(−)-epibatidine showed both a fast and slower uptake and an increasing brain activity with a time constant of about 40 s, the (+) enantiomer showed only a transient peak and a slow decay (Fig. 6). Washout of the ligand could not be determined on this fast time scale measurement. A typical washout time constant of 3.8 h was measured with single photon emission computed tomography for radioiodinated epibatidine cerebellum clearance in baboon [15]. The slowest clearance measured by these authors for this compound was in the thalamus with a clearance time of 11 h.

In the toxicity measurements no deaths occurred in the animal population with the two lower doses (0.04, 0.4 μg/kg for rats and 0.05, 0.5 μg/kg for mice), while many animals died at the two higher doses (8, 80 μg/kg for rats and 10, 100 μg/kg for mice). The LD_{50} in rats was 20 μg/kg (95% confidence limits 8–180 μg/kg) and for mice 15 μg/kg (95% confidence limits 3–120 μg/kg). To break the data down for males and females makes no statistical sense. The clinical signs of toxicity observed in the two high dose groups included sedation, convulsions, ventral recumbency and dyspnea indicating an effect on the nerve system. No clinical signs were observed in the low dose groups. No organ abnormalities were observed on necroscopy.

Thus, it seems that the toxicity of *N*-methylepibatidine is possibly somewhat less than that of the analogous *N*-methylnorchlorofluoroepibatidine [29]. Although adjunction of a methyl side chain to epibatidine reduces the toxic effects somewhat, this is still not sufficient to ensure the complete

Table 1
Sensitivity of epibatidine and derivatives to rat α4β2, α3β4 and α7 nAChRs

cDNA type	(+)-Epibatidine		Methyl-(+)-Epibatidine		(−)-Epibatidine		Methyl-(−)-Epibatidine	
	EC_{50} (μM)	n_H	EC_{50} (μM)	n_H	EC_{50} (μM)	n_H	EC_{50} (μM)	n_H
Rat α7	2.5 ± 016	1.45 ± 0.1	30 ± 0.24	1.60 ± 0.2	2.03 ± 0.5	1.43 ± 0.2	2.5 ± 0.26	2.73 ± 0.2
Rat α4β2	0.021 ± 0.005	1.24 ± 0.1	0.36 ± 0.11	0.45 ± 0.1	0.023 ± 0.007	1.30 ± 0.1	0.024 ± 0.008	1.25 ± 0.1
Rat α3β4	0.036 ± 0.013	1.52 ± 0.4	1.1 ± 0.2	1.60 ± 0.1	0.019 ± 0.006	1.67 ± 0.2	0.035 ± 0.017	1.70 ± 0.1

Half activation values (EC_{50}) and Hill coefficients for three receptor subtypes of the four chemical compounds tested are indicated. Values are the means of 3–7 cells with their respective S.E.M.

safety required for human administration, which is in agreement with the conclusions by Horti et al. [16]. Thus, although indicating the feasibility of the method, new epibatidine derivatives await to be designed to accommodate the properties required both for PET and for human safety.

These data illustrate, however, the importance of isolating the enantiomers and of physiological testing for their characterization.

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References

[1] Langley, J.N. (1905) J. Physiol. 33, 374–413.
[2] Ascher, P., Large, W.A. and Rang, H.P. (1979) J. Physiol. 295, 139–170.
[3] Benowitz, N.L. (1996) Annu. Rev. Pharmacol. Toxicol. 36, 597–613.
[4] Corrigall, W.A., Coen, K.M. and Adamson, K.L. (1994) Brain Res. 653, 278–284.
[5] Cimino, M., Marini, P., Colombo, S., Andena, M., Cattabeni, F., Fornasari, D. and Clementi, F. (1995) J. Neural Transm. Gen. Sect. 100, 77–92.
[6] Deltoro, E.D., Juiz, J.M., Smillie, F.I., Lindstrom, J. and Criado, M. (1997) Dev. Brain Res. 98, 125–133.
[7] Wada, E., Wada, K., Boulter, J., Deneris, E., Heinemann, S., Patrick, J. and Swanson, L.W. (1989) J. Comp. Neurol. 284, 314–335.
[8] Nitta, A., Katono, Y., Itoh, A., Hasegawa, T. and Nabeshima, T. (1994) Pharmacol. Biochem. Behav. 49, 807–812.
[9] Balfour, D.J. (1994) Addiction 89, 1419–1423.
[10] Zoli, M., Le, N.N., Hill, J.J. and Changeux, J.P. (1995) J. Neurosci. 15, 192–1939.
[11] Nyback, H., Halldin, C., Ahlin, A., Curvall, M. and Eriksson, L. (1994) Psychopharmacology (Berl.) 115, 31–36.
[12] Muric, R.F., Berridge, M.S., Friedland, R.P., Zhu, N. and Nelson, A.D. (1998) J. Nucl. Med. 39, 2048–2054.
[13] Patt, J.T., Westera, G., Buch, A., Fletcher, S.R. and Schubiger, P.A. (1995) J. Lab. Comp. Radiopharm. 17, 355–356.
[14] Villemagne, V.L. et al. (1997) J. Nucl. Med. 38, 1737–1741.
[15] Musachio, J.L., Villemagne, V.L., Scheffel, U., Stathis, M., Finley, P., Horti, A., London, E.D. and Dannals, R.F. (1997) Synapse 26, 392–399.
[16] Horti, A.G. et al. (1998) J. Med. Chem. 41, 4199–4206.
[17] Kassiou, M., Scheffel, U.A>, Ravert, H.T., Mathews,, W.B., Musachio, J.L., London, E.D. and Dannals, R.F. (1998) Life Sci. 63, L13–18.
[18] Patt, J.T., Spang, J.E., Westera, G., Buck, A. and Schubiger, P.A. (1999) Nucl. Med. Biol. (in press).
[19] Bertrand, D., Cooper, E., Valera, S., Rungger, D. and Ballivet, M. (1991) Methods Neurosci. 4, 174–193.
[20] Bertrand, D. and Changeux, J.P. (1995) Semin. Neurosci. 7, 75–90.
[21] Boyd, R.T., Jacob, M.H., McEachern, A.E., Caron, S. and Berg, D.K. (1991) J. Neurobiol. 22, 1–14.
[22] Conroy, W.G. and Berg, D.K. (1995) J. Biol. Chem. 270, 4424–4431.
[23] Séguéla, P., Wadiche, J., Dineley-Miller, K., Dani, J.A. and Patrick, J.W. (1993) J. Neurosci. 13, 596–604.
[24] Alkondon, M. and Albuquerque, E.X. (1995) J. Pharmacol. Exp. Ther. 274, 771–782.
[25] Gerzanich, V., Peng, X., Wang, F., Wells, G., Anand, R., Fletcher, S. and Lindstrom, J. (1995) Mol. Pharmacol. 48, 774–782.
[26] Couturier, S. et al. (1990) Neuron 5, 847–856.
[27] Gopalakrishnan, M. et al. (1995) Eur. J. Pharmacol. Mol. Pharmacol. 290, 237–246.
[28] Scheffel, U., Taylor, G.F., Kepler, J.A., Carroll, F.I. and Kuhar, M.J. (1995) NeuroReport 6, 2483–2488.
[29] Ding, Y.S., Molina, P.E., Fowler, J.S., Logan, J., Volkow, N.D., Kuhar, M.J. and Caroll, F.I. (1999) Nucl. Med. Biol. 26, 139–148.