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Synthesis of (*S*)-*N*-[Methyl-^{11}C]nicotine and Its Regional Distribution in the Mouse Brain: A Potential Tracer for Visualization of Brain Nicotinic Receptors by Positron Emission Tomography

Hideo SAJI,*[a] Yasuhiro MAGATA,[b] Yoshihisa YAMADA,[a] Ken TAJIMA,[a] Yoshiharu YONEKURA,[b] Junji KONISHI,[b] Yoshiro OHMOMO,[c] and Akira YOKOYAMA[a]

Department of Radiopharmaceutical Chemistry, Faculty of Pharmaceutical Sciences,[a] and School of Medicine,[b] Kyoto University, Yoshida Shimoadachi, Sakyo-ku, Kyoto 606-01, Japan and Osaka University of Pharmaceutical Sciences,[c] 2-10-65 Kawai, Matsubara, Osaka 580, Japan.
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A nicotine agonist, ^{11}C-labeled (*S*)-nicotine, was synthesized by N-methylation of (*S*)-nornicotine with [^{11}C]-methyl iodide in dimethylformamide–dimethylsulfoxide in order to study nicotinic receptors in the human brain by positron emission tomography. The radiochemical yield of this N-methylation reaction was more than 90% within 5 min. After purification by high performance liquid chromatography the radiochemical purity of the product was more than 99% and the specific radioactivity was 7.4—11.1 GBq/μmol. The regional distribution of (*S*)-[^{11}C]nicotine in the mouse brain after intravenous injection was compared with that of (*R*)-[^{11}C]nicotine. After injection of (*S*)-[^{11}C]nicotine, the regional uptake of radioactivity was in the following order: cortex > thalamu ≈ hippocampus > striatum > hypothalamus > cerebellum. Moreover, (*S*)-[^{11}C]nicotine was displaced from the brain by unlabeled (*S*)-nicotine, but unlabeled (*R*)-nicotine caused no change in uptake. In contrast, (*R*)-[^{11}C]nicotine showed a lower brain uptake and lesser regional differences in radioactivity.

Keywords carbon-11 labeled nicotine; radiosynthesis; optic enantiomer; central nicotinic receptor; positron emission tomography

Introduction

Nicotine induces a number of physiological effects on the central nervous system which are thought to be mainly mediated *via* nicotinic receptors.[1] Recently, heterogenous nicotinic receptors have been characterized in brain tissue by *in vitro* receptor binding studies and at least two types of nicotinic binding sites have been found, a high affinity site and a low affinity site.[2] A decrease in the number of high affinity nicotinic receptors and a concomitant reduction in the proportion of high affinity to low affinity receptors have been reported in Alzheimer brains.[3] An increased density of nicotinic receptors has also been reported in the brains of smokers.[4] Positron emission tomography (PET), on the other hand, has recently attracted significant attention as a useful tool for studying the neuroreceptors of the living human brain.[5] Thus, research on the imaging and quantitative assessment of brain nicotinic receptors *in vivo* using a suitable radioligand and PET is currently attracting great interest.

The basic requirements for a radioligand to be useful for *in vivo* PET studies of neurotransmitter receptors include a high affinity to a specific receptor as well as rapid and quantitatively significant brain uptake following peripheral administration.[6] Nicotine has two stereoisomers, (*S*)- and (*R*)-nicotine, due to the presence of a center of asymmetry at the carbon joining the pyrrolidine ring to the pyridine moiety. The (*S*)-enantiomer, which is the naturally occurring form of nicotine, has a greater affinity for nicotinic receptors in the brain than the (*R*)-enantiomer.[7] Furthermore, nicotine passes instantaneously through the blood–brain barrier (BBB).[8] Thus, we selected ^{11}C-labeled (*S*)-nicotine as a radioligand for the *in vivo* nicotinic receptor studies using PET. Although (*S*)-[^{11}C]nicotine has already been synthesized by other research groups,[9] no report has appeared on its detailed regional distribution in the brain as compared with that of the (*R*)-enantiomer.

In this paper, we described the synthesis of (*S*)- and (*R*)-[^{11}C]nicotine, their regional brain distribution in mice, and the effect of unlabeled (*S*)- and (*R*)-nicotine on their cerebral uptake and distribution.

Materials and Methods

(*S*)- and (*R*)-nornicotine were obtained from Wako Pure Chemical Industries, Ltd. Unlabeled (*R*)-nicotine was kindly supplied by Japan Tobacco Company. The other chemicals used were of reagent grade. Male ddY mice were supplied by Japan SLC Co., Ltd.

Synthesis of (*S*)- and (*R*)-[^{11}C]Nicotine (Fig. 1) (*S*)- and (*R*)-[^{11}C]-nicotine were synthesized using the method of Langstrom *et al.* with a slight modification.[10]

$^{11}CO_2$ was produced *via* proton bombardment of nitrogen gas by the $^{14}N(p, \alpha)^{11}C$ reaction using an ultra-compact cyclotron (Sumitomo, model 325), and was trapped in a solution of $LiAlH_4$ in tetrahydrofuran (THF). After evaporation of the THF, 54% hydroiodic acid was added, and the [^{11}C]methyl iodide produced was trapped in dimethylformamide (DMF) under a stream of nitrogen gas. Then, 3 mg of (*S*)- or (*R*)-nornicotine was dissolved in 300 μl of dimethylsulfoxide (DMSO)–DMF (200 : 100, v/v) in a reaction vial, and 300 μl of DMF containing [^{11}C]methyl iodide was added to the vial. The vial was heated at 115 °C for 5 min, and the resulting mixture was purified by preparative high performance liquid chromatography (HPLC) on a 7.5 × 300 mm Partisil 5 column eluted with $CHCl_3$–C_2H_5OH–$(C_2H_5)_3N$ (2400 : 97 : 3) at 2 ml/min. The fraction corresponding to nicotine was corrected and evaporated. The residue was then dissolved in phosphate-buffered saline (pH 7.4), and filtered through a 0.22 μm filter. Radiochemical purity was determined by analytical HPLC using a 4.6 × 250 mm Partisil 10-SCX column eluted with methanol–0.3 M acetate buffer (pH 4.5) (30 : 70) at 1 ml/min (t_R = 12.2 min for nicotine, t_R = 9.3 min for nornicotine, and t_R = 4.2 min for methyl iodide). The specific activity was estimated by the ultraviolet (UV) absorbance at 254 nm.

Regional Cerebral Distribution in the Mouse Brain Male mice weighing an average of 30 g were injected intravenously with 0.1 ml of a solution of (*S*)- or (*R*)-[^{11}C]nicotine (15 MBq). At various times after the injection, the mice were killed by decapitation, and the various brain regions were dissected out on an ice-cold plate according to the method of Glowinski and Iversen.[11] The wet tissue samples were then weighed, and the radioactivity was determined using a well-type NaI scintillation counter. Results were calculated in terms of the percentage of the injected dose per gram of tissue.

Drug Displacement Studies Unlabeled (*S*)- or (*R*)-nicotine (60 μg/kg)

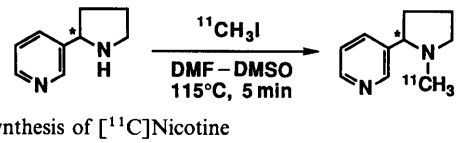

Fig. 1. Synthesis of [^{11}C]Nicotine

*: Asymmetric carbon.

was injected simultaneously with the radioligand into mice weighing 30 g. The animals were killed 30 min after administration and the uptake of the radioligand by various brain regions was determined as described above.

Results

Synthesis of (*S*)- and (*R*)-[^{11}C]Nicotine (*S*)- and (*R*)-[^{11}C]nicotine were synthesized by N-methylation of the corresponding nornicotine with [^{11}C]methyl iodide in DMF–DMSO. The radiochemical yield of this N-alkylation reaction was more than 90%, and the total time from the end of [^{11}C]methyl iodide trapping to HPLC purification was under 25 min. The radiochemical purity of the (*S*)- and (*R*)-[^{11}C]nicotine thus obtained was more than 99% and the specific radioactivity was 7.4—11.1 GBq/μmol. (*S*)- and (*R*)-[^{11}C]nicotine were also synthesized by N-methylation in DMF using a base like tetrabutylammonium hydroxide, but the radiochemical yield of this reaction was about 1.5—2 times less than that achieved with DMF–DMSO.

Regional Cerebral Distribution The time course of changes in the distribution of (*S*)- and (*R*)-[^{11}C]nicotine radioactivity in the blood and various brain regions is shown in Table I.

(*S*)-[^{11}C]Nicotine rapidly entered the brain, and a high uptake of radioactivity was observed at the initial sampling time of 5 min, after which it declined with time. Differences in the distribution of radioactivity were observed between the various brain regions: *i.e.*, the cortex showed the highest uptake followed by hippocampus, thalamus, striatum, hypothalamus, and cerebellum in that order. The blood radioactivity was cleared rapidly and the level at 5 min was far lower than that reached in the brain.

In contrast, injection of (*R*)-[^{11}C]nicotine produced a lower uptake of radioactivity by the brain and smaller regional differences than (*S*)-[^{11}C]nicotine, although both enantiomers had a similar blood radioactivity level.

Effect of (*S*)- and (*R*)-Nicotine on Regional Cerebral Distribution The effects of treatment with unlabeled (*S*)- or (*R*)-nicotine (60 μg/kg) on the cerebral distribution of both ^{11}C-labeled enantiomers at 30 min after injection are shown in Fig. 2. Treatment with (*S*)-nicotine tended to reduce the uptake of (*S*)-[^{11}C]nicotine in all brain regions tested, although uptake was still not significantly different from that obtained without (*S*)-nicotine (8 to 20%) reduction). However, (*R*)-nicotine caused no changes in uptake in most brain regions, although that in the hippocampus was somewhat depressed. In contrast, the brain uptake of (*R*)-[^{11}C]nicotine was not affected by the administration of unlabeled (*S*)- or (*R*)-nicotine.

TABLE I. Biodistribution of (*S*)- and (*R*)-[^{11}C]Nicotine in Blood and Various Brain Regions in Mice (% Dose/g)

Region	Time after injection (min)			
	5	15	30	60
(*S*)-[^{11}C]Nicotine				
Blood	1.93 (0.21)[a]	1.48 (0.18)	1.49 (0.24)	1.30 (0.12)
Cortex	7.04 (0.68)	2.94 (0.67)	2.20 (0.27)	1.40 (0.12)
Striatum	6.03 (0.61)	2.64 (0.64)	1.99 (0.28)	1.34 (0.09)
Hippocampus	6.41 (0.49)	3.04 (0.56)	2.21 (0.36)	1.39 (0.16)
Thalamus	6.38 (0.71)	2.79 (0.64)	2.17 (0.25)	1.38 (0.21)
Hypothalamus	5.82 (0.40)	2.67 (0.59)	2.04 (0.36)	1.32 (0.24)
Cerebellum	4.68 (0.40)	2.22 (0.44)	1.75 (0.16)	1.20 (0.08)
(*R*)-[^{11}C]Nicotine				
Blood	2.29 (0.14)	2.04 (0.07)	1.36 (0.21)	0.74 (0.08)
Cortex	4.92 (0.76)	1.73 (0.06)	0.88 (0.08)	0.52 (0.06)
Striatum	4.17 (0.64)	1.48 (0.10)	0.76 (0.04)	0.42 (0.08)
Hippocampus	4.53 (0.63)	1.78 (0.14)	0.86 (0.04)	0.49 (0.08)
Thalamus	4.28 (0.70)	1.49 (0.06)	0.73 (0.14)	0.45 (0.07)
Hypothalamus	3.82 (0.46)	1.50 (0.05)	0.79 (0.06)	0.59 (0.08)
Cerebellum	3.35 (0.50)	1.30 (0.05)	0.77 (0.06)	0.50 (0.05)

a) Each value is the mean (±S.D.) of 4 animals.

Discussion

In this study, (*S*)-[^{11}C]nicotine was evaluated as a radioligand for the investigation of brain nicotinic receptors by PET.

After intravenous administration, (*S*)-[^{11}C]nicotine showed rapid uptake by the brain and then washed out with time (Table I). The time course of the changes in ^{11}C brain radioactivity following (*S*)-[^{11}C]nicotine administration was similar to that reported by Maziere *et al.* and Nordberg *et al.*[9] This rapid decline may be related to the short drug–receptor interaction time of (*S*)-nicotine.[12]

Regarding the metabolism of this compound, Appelgren *et al.* reported that only small amounts of cotinine, a major metabolite of nicotine, were found in the brain following the intravenous injection of [^{14}C]nicotine into mice.[13] Their observation suggests that the major part of the radioactivity found in the brain in this study was in the form of [^{11}C]nicotine itself.

After the injection of (*S*)-[^{11}C]nicotine, the uptake of radioactivity was in the following order: cortex > hippo-

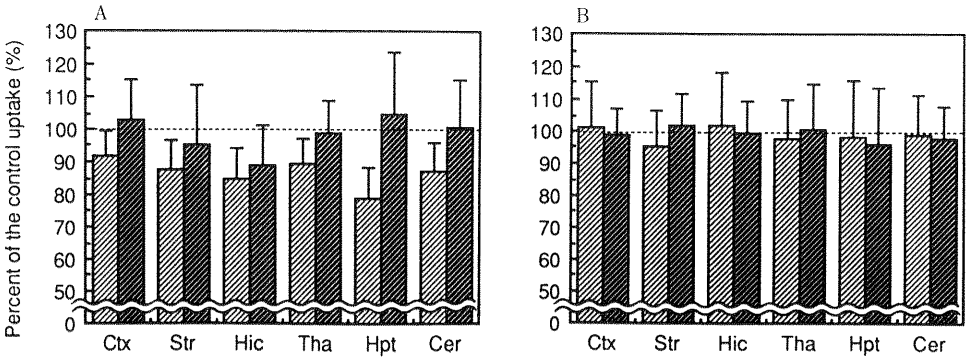

Fig. 2. Effects of Unlabeled (*S*)- and (*R*)-Nicotine on the Regional Cerebral Distribution of (*S*)- and (*R*)-[^{11}C]Nicotine in Mice

A, (*S*)-[^{11}C]nicotine; B, (*R*)-[^{11}C]nicotine. ▨, (*S*)-nicotine; ■, (*R*)-nicotine. Ctx, cortex; Str, striatum; Hic, hippocampus; Tha, thalamus; Hpt, hypothalamus; Cer, cerebellum.

campus ≈ thalamus > striatum > hypothalamus > cerebellum (Table I). This regional distribution agrees well with the results obtained by the *in vitro* mapping of nicotinic receptors.[14] Furthermore, treatment with unlabeled (*S*)-nicotine, an active form of nicotine,[7] reduced the brain uptake of (*S*)-[^{11}C]nicotine, while the less potent (*R*)-nicotine caused almost no changes in uptake (Fig. 2). Thus, a stereospecificity of the cerebral binding of (*S*)-[^{11}C]nicotine was demonstrated by the competition experiment performed with the two unlabeled isomers of nicotine. (*R*)-[^{11}C]Nicotine, on the contrary, showed a far lower brain uptake (Table I). Since (*R*)-[^{11}C]nicotine had a similar blood radioactivity level to that of (*S*)-[^{11}C]nicotine, there may have been no difference in the radioactivity supplied from the blood to the brain by these two ^{11}C-labeled enantiomers. Therefore, the low brain uptake of (*R*)-[^{11}C]nicotine is presumed to be due to a lack of any specific interaction which allows it to be retained in the brain tissue. Moreover, (*R*)-[^{11}C]nicotine showed less regional differences in brain radioactivity and no changes in uptake following the administration of either unlabeled (*S*)- or (*R*)-nicotine (Table I, Fig. 2). The differences between (*S*)- and (*R*)-[^{11}C]nicotine shown by these *in vivo* cerebral distribution and competition studies indicate that (*S*)-[^{11}C]nicotine binds to brain nicotinic receptors.

However, the administration of (*S*)-nicotine prevented the uptake of (*S*)-[^{11}C]nicotine in all cerebral regions tested, including the cerebellum, a region of low nicotinic receptor density.[14] Although the cause of this finding is not certain at present, a similar phenomenon has been noted for [^{11}C]deprenorphine binding to opiate receptors and was explained by partial receptor saturation.[15] This explanation might also apply to (*S*)-[^{11}C]nicotine.

In conclusion, the results obtained in this study indicate that (*S*)-[^{11}C]nicotine binds to nicotinic receptors in the brain following intravenous injection. (*S*)-[^{11}C]Nicotine therefore appears to be a radiopharmaceutical with potential for use in the investigation of central nicotinic receptors in the human brain.

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References

1) A. Nordberg, K. Fuxe, B. Holmstedt, and A. Sundwall (eds.), "Nicotinic Receptors in the CNS: Their Role in Synaptic Transmission," Elsevier, Amsterdam, 1989.
2) S. Wonnacott, "Nicotine Psychopharmacology," ed. by S. Wonnacott, M. A. H. Russell, and I. P. Stolerman, Oxford University Press, New York, 1990, pp. 226—277; E. Giacobini, *J. Neurosci. Res.*, **27**, 548 (1990).
3) A. Nordberg and B. Winblad, *Neurosci. Lett.*, **72**, 115 (1986); A. Nordberg, A. Adem, J. Hardy, and B. Winblad, *ibid.*, **86**, 317 (1988).
4) M. E. M. Benwell, D. J. K. Balfour, and J. M. Anderson, *J. Neurochem.*, **50**, 1243 (1988).
5) J. J. Frost and H. N. Wagner, Jr., "Quantitative Imaging," Raven Press, New York, 1990.
6) C. D. Arnett, C-Y. Shiue, A. P. Wolf, J. S. Fowler, J. Logan, and M. Watanabe, *J. Neurochem.*, **44**, 835 (1985); H. F. Kung, *Nucl. Med. Biol.*, **17**, 85 (1990).
7) A. M. Martino-Barrows and K. J. Kellar, *Mol. Pharmacol.*, **31**, 169 (1987); C. Reavill, P. Jenner, R. Kumar, and I. P. Stolerman, *Neuropharmacology*, **27**, 235 (1988).
8) A. Tsujimoto, T. Nakashima, S. Tanino, T. Dohi, and Y. Kurogochi, *Toxicol. Appl. Pharmacol.*, **32**, 21 (1975); W. H. Oldendorf, *Exp. Eye Res.*, **1977**, Suppl., 177.
9) M. Maziere, D. Comar, C. Marazano, and G. Berger, *Eur. J. Nucl. Med.*, **1**, 255 (1976); A. Nordberg, P. Hartvig, H. Lundqvist, G. Antoni, J. Ulin, and B. Langstrom, *J. Neural Transm.* [P-D Sect.], **1**, 195 (1989).
10) B. Langstrom, G. Antoni, C. Halldin, H. Svard, and G. Bergson, *Chemica Scripta*, **20**, 46 (1982).
11) J. Glowinski and L. L. Iversen, *J. Neurochem.*, **13**, 655 (1966).
12) C. Larsson and A. Nordberg, *J. Neurochem.*, **45**, 24 (1985).
13) L. E. Appelgren, E. Hansson, and C. G. Schmiterlow, *Acta Physiol. Scand.*, **56**, 249 (1962).
14) K. Yoshida and H. Imura, *Brain Res.*, **172**, 453 (1979); P. B. S. Clarke, R. D. Schwartz, S. M. Paul, C. B. Pert, and A. Pert, *J. Neurosci.*, **5**, 1307 (1985).
15) B. Sadzot, J. C. Price, H. S. Mayberg, K. H. Douglass, R. F. Dannals, J. R. Lever, H. T. Ravert, A. A. Wilson, H. N. Wagner, Jr., M. A. Feldman, and J. J. Frost, *J. Cereb. Blood Flow Metab.*, **11**, 204 (1991).