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Uptake and regional distribution of (+)-(R)- and (−)-(S)-N-[methyl-^{11}C]-nicotine in the brains of Rhesus monkey An attempt to study nicotinic receptors in vivo

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Summary. N-[methyl-^{11}C] nicotine (^{11}C-nicotine) was given intravenously to monkeys and the uptake and regional distribution of radioactivity was followed in the brain using positron emission tomography (PET). The ^{11}C-radioactivity in the brain peaked within 1–2 min and then rapidly declined. Pretreatment with unlabelled nicotine (10 μg/kg) reduced the uptake of ^{11}C-radioactivity to the brain by 30%. The uptake of radioactivity was higher following (+)^{11}C-nicotine than (−)^{11}C-nicotine. Both enantiomers were distributed in a similar manner within the brain. When animals were infused with a peripheral nicotinic blocker (trimetaphan) the uptake of radioactivity to the brain was lower following (+)^{11}C-nicotine compared to (−)^{11}C-nicotine. The amount of radioactivity was high in the occipital cortex, thalamus, intermediate in the frontal cortex and low in white matter in (−)^{11}C injected monkeys while no regional difference in distribution of ^{11}C-radioactivity was observed after injection of (+)^{11}C-nicotine.

Keywords: Monkey, positron emission tomography, ^{11}C-nicotine, optic enantiomers, nicotinic receptors, brain, regional distribution.

Introduction

The widespread use of tobacco is largely assumed to reflect the effect of nicotine as a primary reinforcer. Nicotine induces both in experimental animals and man a number of physiological effects (Mangan and Golding, 1985). The underlying mechanisms for the effects of nicotine in brain are still relatively unknown but it is assumed that the effects are mainly mediated via nicotinic receptors. During recent years heterogenous nicotinic binding sites have been characterized by in vitro receptor binding studies in brain tissue (for reviews,

see Larsson, 1985; Adem, 1987; Wonnacott, 1987). At least two types of nicotinic binding sites, a high affinity site and a low affinity site have been measured using radiolabelled nicotinic agonists such as ^{3}H-nicotine and ^{3}H-acetylcholine. In in vitro binding studies to tissue homogenates as well as to thin tissue slices (autoradiography) the nicotinic binding sites have showed a similar regional distribution both in rodent (Larsson and Nordberg, 1985; Härfstrand et al., 1988) and human brain (Nordberg et al., 1988 b, c; Adem and Nordberg, 1988; Adem et al., 1988 a, b). The number of high affinity nicotinic binding sites varies between 50–100 pmol/g protein depending what region that is studied. Following repeated administration of nicotine to rodents an increased number of high affinity nicotinic receptors has been measured (Schwartz and Kellar, 1983; Larsson et al., 1986). The increase in number of high affinity sites might be due to an interconversion of low to high affinity sites (Romanelli et al., 1988). An increased number of nicotinic receptors has also recently been reported in postmortem brains of smokers (Benwell et al., 1988).

Positron emission tomography (PET) enables in vivo visualization of different receptors populations in the brain (Phelps et al., 1986; Greitz et al., 1985; Hayaishi and Torizuka, 1986). In order to study nicotinic receptors in vivo we have administered N-[methyl-^{11}C] nicotine (^{11}C-nicotine) intravenously to monkeys and followed the uptake and regional distribution of radioactivity in the brain. Racemic ^{11}C-nicotine and the two enantiomers (−)-(S)-and (+)-(R)^{11}C-nicotine were used. A difference in the regional distribution of ^{11}C-radioactivity was observed in brain following injection of (−)^{11}C-nicotine and (+)^{11}C-nicotine during blockade of the peripheral cholinergic nicotinic receptors by trimetaphan.

Material and methods

Synthesis of ^{11}C-labelled nicotine

Racemic ^{11}C-nicotine, (−)-(S)^{11}C-nicotine, and (+)-(R)^{11}C-nicotine were synthesized using either racemic nornicotine, or (−) and (+) nornicotine and according to earlier described methods (Långström et al., 1982) with some minor modifications (Långström et al., 1987). The specific radioactivity was 10–100 mCi/μmol. (+)-(R)- and (−)-(S)nornicotine were kindly supplied by Dr. Peyton Jacob, Div. of Clinical Pharmacology, University of California, San Francisco, CA, U.S.A. The enantiomers had a purity of > 95% (Jacob, 1982).

In a typical synthesis 3–4 mg of the appropriate hydrochloride of nornicotine was dissolved in 500 μl of dimethylsulfoxide (DMSO)/dimethylformamide (DMF) (200/300 v/v) together with 5 μl of tetramethylpiperidine (TMP) in a 1 ml reaction vial. ^{11}C-Methyl iodide, produced by the procedure at our laboratory described in detail elsewhere (Långström et al., 1987) was then trapped in the vial and kept at 100 °C for 5 min. The reaction was stopped by addition of 500 μl of the LC-eluent and applied onto the preparative LC-column. LC-purification was performed on a 250 × 10 mm reversed phase C-18 Nucleosil (30 μm) using ethanol/0.05 M ammonium formiate (13/87 v/v) at a flow of 6 ml/min. The appropriate radioactive fractions were collected and evaporated. The residue was then dissolved in phosphate buffer and saline and filtered through a 0.22 μm filter before injected. Radiochemical and chemical purity was determined by analytical LC using a 250 × 4.6 mm reversed phase Alltech (10 μm) using methanol/0.05 M ammonium formiate (55/45 v/v) at a flow of 2 ml/min using a UV-diode array detector at a wavelength of 250 nm in series with a radioflow detector (Långström and Lundqvist, 1979).

Animals

Female Rhesus monkeys (*Macacca Mulatta*) weighing 6–10 kg from the Primate Laboratory of Reproductive Research at the University of Uppsala, were used after an overnight fast. The monkeys were anaesthetized with 100 mg of ketamine (Ketalar®, Parke-Davis) and ketamine in doses of 50–100 mg and diazepam (Diazemuls®) in doses of 50–100 mg were subsequently given intramuscular as required. A venous catheter was inserted in each hind leg of the monkey, one for injection of drugs and radioactive dose and one for blood sampling. The monkey was positioned in the tomograph so that the lowest horizontal transection of the head included the cerebellum and basal parts of temporal lobes. The ^{11}C-nicotine was given intravenously as a bolus.

Baseline experiments

Seven monkeys were receiving intravenous injections of racemic, (+)-(R)- or (−)-(L) ^{11}C-nicotine. The radioactive dose of ^{11}C-nicotine used was in the order of 16–150 MBq.

Pretreatment experiments

In one set of experiments 10 μg/kg of unlabelled nicotine bitartrate (racemic form, calculated as base) was given intravenously 2 min before injection of racemic ^{11}C-nicotine. In a second set of experiments the nicotinic antagonist mecamylamine in a dose of 0.4 mg/kg was given seven min before injection of racemic ^{11}C-nicotine. In a third set of experiments trimetaphan (Arfonad®) was given as an intravenous infusion (0.1 mg/ml/min) starting 10 min before injection of (+)^{11}C-nicotine and (−)^{11}C-nicotine, respectively. The infusion ended 25 min after the injection of ^{11}C-nicotine. In all experiments (+)^{11}C-nicotine was injected in the first study and (−)^{11}C-nicotine in the second study on the same day.

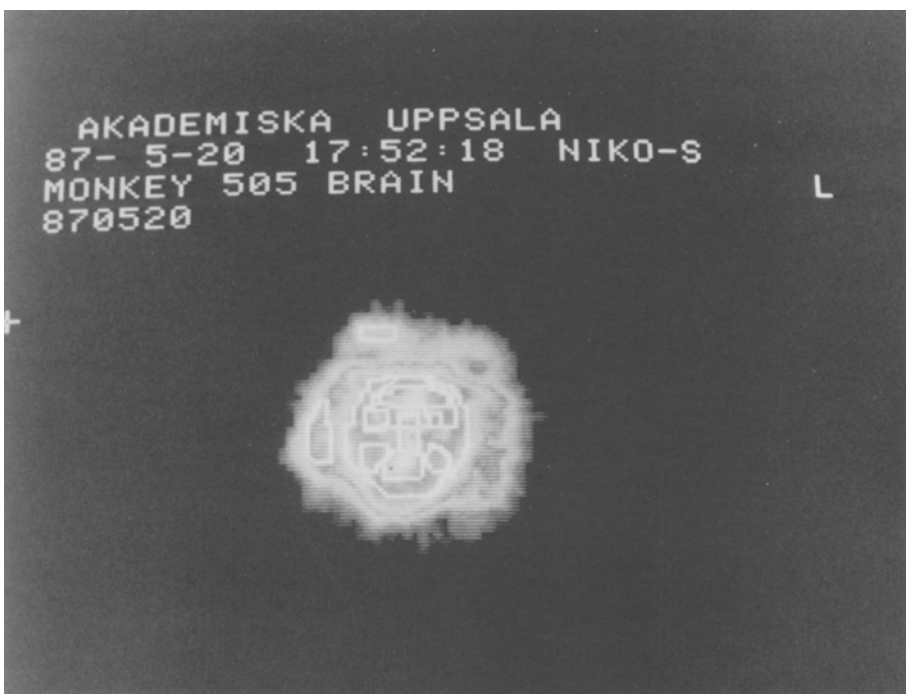

Fig. 1. Localization of different Roi's chosen in the monkey head. The size of each region varies between 10–80 pixel. One pixel corresponds to 2.5 × 2.5 mm

Analysis with positron emission tomography

Immediately following administration of the radioactive dose of ^{11}C-nicotine to the monkey, the PC 384-3B positron emission tomograph (AB Scanditronix, Uppsala, Sweden) positioned over the brain was started. Images were recorded for 12, 40, 600 s at determined periods for 60–90 min and reconstructed according to Eriksson et al. (1982). The resolution of the camera was 0.8 cm. Figure 1 illustrates the localization of the Roi's that were chosen. The size of each region varied between 10–80 pixel. One pixel corresponds to 2.5 × 2.5 mm. To decide the anatomical position of each Roi the localization was compared with that in a serial cryosectioned monkey head. The following regions of interest in the head of the Rhesus monkey were analysed for radioactivity: frontal, temporal, and occipital cortex, thalamus, cerebellum, white matter, total brain, muscle, eye. The obtained radioactivity values were corrected for physical decay. The relative distribution of the radioactivity in different tissues was calculated from the corrected radioactivity per cm^3 and divided by the radioactive dose per g body weight.

Results

The uptake of ^{11}C-radioactivity to total brain, eye, muscle, as measured by PET, after intravenous administration of racemic ^{11}C-nicotine to Rhesus monkey is shown in Fig. 2. The ^{11}C-radioactivity in the brain peaked within 1–2 min and then rapidly declined with an initial half life of about 12 min followed by a slower decline with a half life of more than 2 h. The uptake of ^{11}C-radioactivity in muscle was much lower than in the brain and remained constant for 80 min. A rather high and stable concentration of ^{11}C-radioactivity (twice the value for muscle) was observed in a region including the eye (Fig. 2).

The time course of ^{11}C-radioactivity when racemic ^{11}C-nicotine was given to monkeys pretreated with nicotine (10 μg/kg) or the nicotine receptor antagonist mecamylamine (0.4 mg/kg) are shown in Fig. 3. Pretreatment with unlabelled nicotine reduced the uptake of ^{11}C-radioactivity to the brain by 30 per cent. In mecamylamine pretreated animals on the other hand, the uptake of ^{11}C-radioactivity to the brain was slightly higher during first 15 min compared to controls. In the region including muscle (Fig. 3) pretreatment with both

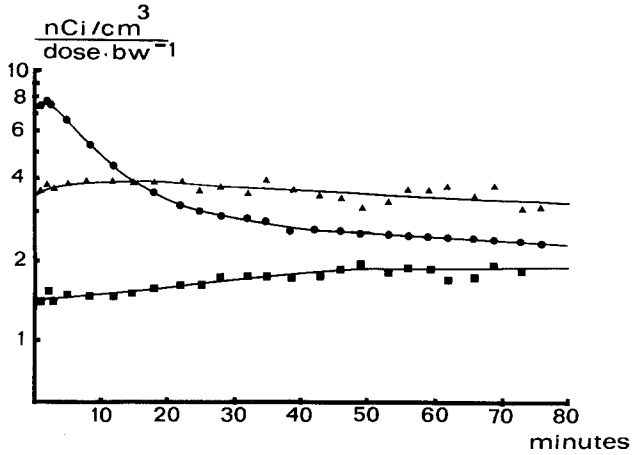

Fig. 2. Uptake and time course of ^{11}C-radioactivity in brain (●), eye (▲), and muscle (■) as measured by PET after intravenous injection of racemic ^{11}C-nicotine to Rhesus monkey

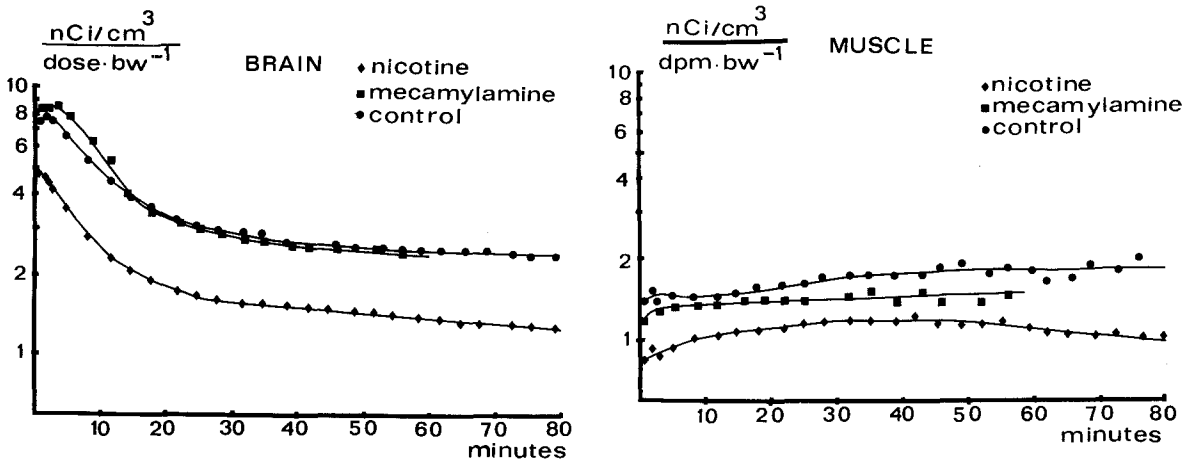

Fig. 3 a, b. Time course of ^{11}C-radioactivity in brain (**a**) and muscle (**b**) following intravenous injection of racemic ^{11}C-nicotine to monkeys pretreated with nicotine (10 μg/kg) (◆) or mecamylamine (0.4 mg/kg) (■). ● Control

nicotine and mecamylamine reduced the uptake of ^{11}C-radioactivity. The uptake of radioactivity in monkey brain was higher following (+)^{11}C-nicotine as compared to (−)^{11}C-nicotine (Fig. 4 a, b). The both enantiomers, however, were distributed in similar manner in the brain. Thus, a high amount of radioactivity was observed in the cortex compared to cerebellum. Following both enantiomers the amount of radioactivity in the region including the "eye" and the muscle (extracranial tissue) was low compared to the brain, as earlier observed for the racemic ^{11}C-nicotine (Fig. 2). The amount of ^{11}C-radioactivity in the blood was lower than in the brain and decreased rapidly, and very similarly following (−)^{11}C-nicotine and (+)^{11}C-nicotine respectively (Fig. 5). In monkeys infused with a peripheral ganglionic blocker (trimetaphan) the uptake of radioactivity to the brain was lower following (+)^{11}C-nicotine compared to (−)^{11}C-nicotine. No regional difference in the distribution of radioactivity was observed in the brain following intravenous injection of (+)^{11}C-nicotine (Fig. 4 c) while it was observed for the (−)isomer (Fig. 4 d). Thus, following (−)^{11}C-nicotine the amount of radioactivity was high in the occipital cortex, thalamus, intermediate in the frontal cortex and low in the white matter. Figure 6 illustrates the difference in uptake of ^{11}C-radioactivity observed in the temporal cortex following injection of (−)^{11}C-nicotine and (+)^{11}C-nicotine to monkeys under continuous infusion of a peripheral nicotinic receptor blocker (trimetaphan).

Discussion

In this study ^{11}C-nicotine given intravenously in a bolus dose was rapidly distributed to the brain and then followed by a rapid decline in radioactivity. The time course of ^{11}C-radioactivity in brain following ^{11}C-nicotine was similar to that reported by Stålhandske (1970) following intravenous ^{14}C-nicotine injections in mice and by Maziere et al. (1969) following (−)^{11}C-nicotine injections

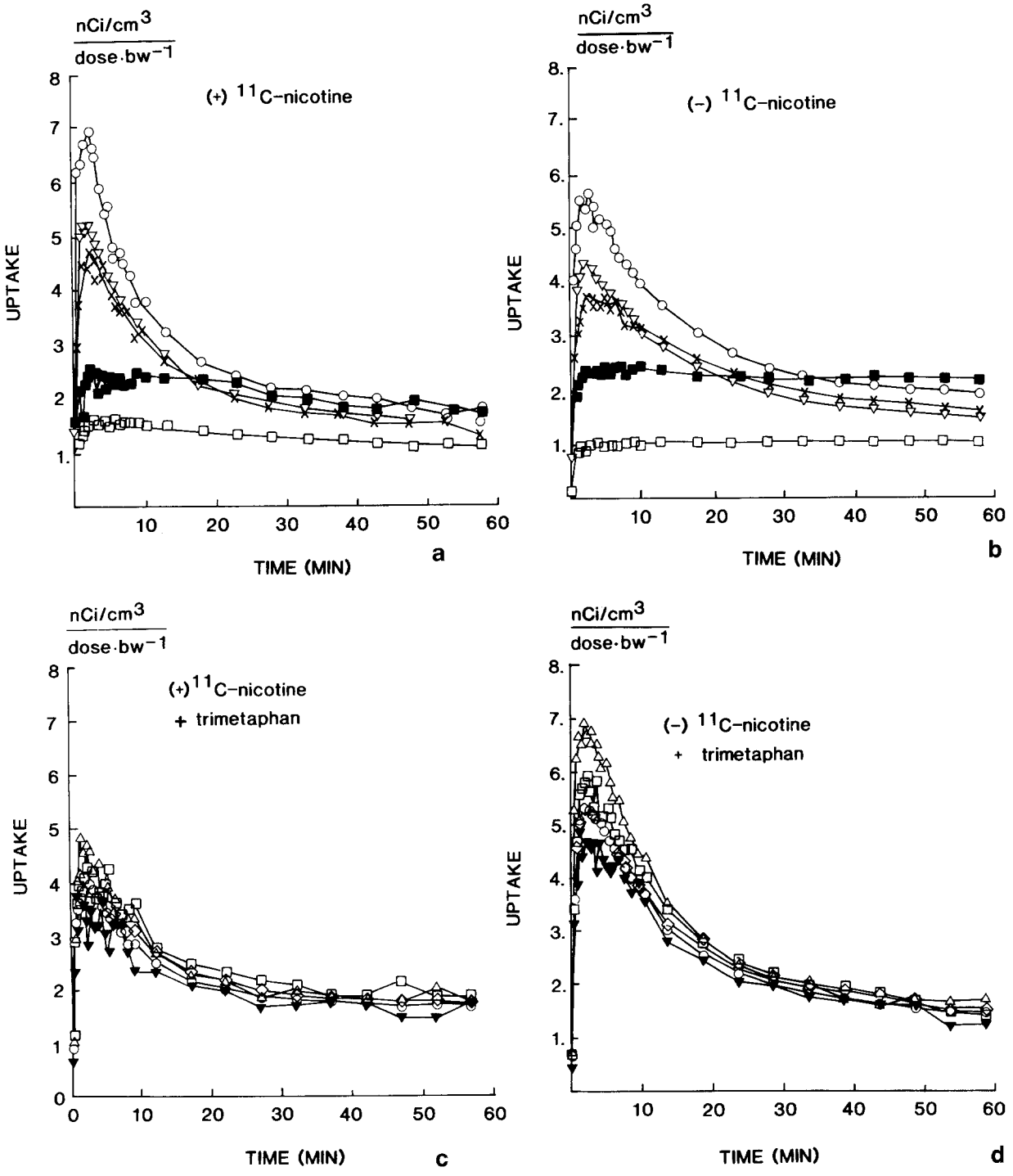

Fig. 4a–d. Uptake and time course of ^{11}C-radioactivity in different brains regions following injection of (+)^{11}C-nicotine and (—)^{11}C-nicotine to monkeys in the absence (**a, b**) and presence (**c, d**) of a peripheral nicotinic blocker (trimetaphan). **a, b** ○ Frontal cortex, ▽ total brain, × cerebellum, ■ eye, □ extracranial tissue. **c, d** △ Thalamus, □ occipital cortex, ◇ frontal cortex, ○ total brain, ▼ white matter; trimetaphan was given as an intravenous infusion (0.1 mg/ml/min) starting 10 min before injection of ^{11}C-nicotine

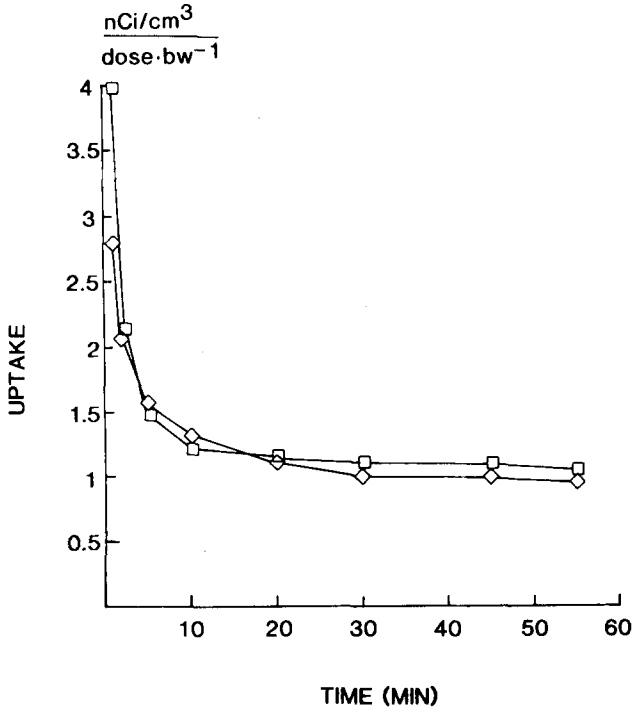

Fig. 5. Time course of ^{11}C-radioactivity in blood following intravenous injection of (+)^{11}C-nicotine (◇) and (—)^{11}C-nicotine (□) to Rhesus monkey

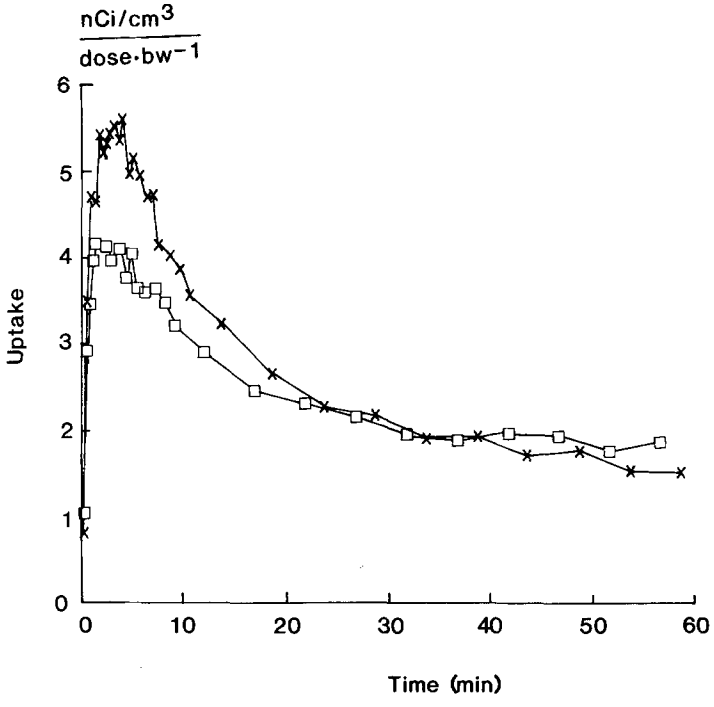

Fig. 6. Time course of ^{11}C-radioactivity in the frontal cortex following intravenous injection of (+)^{11}C-nicotine (□) and (—)^{11}C-nicotine (×) in monkeys receiving a continuous infusion of the ganglionic blocker trimetaphan (0.1 mg/ml/min)

to monkeys. In mice cotinine, the main metabolite of nicotine, has been reported to constitute less than 20 per cent of the radioactivity in brain 15 min after intravenous injection of ^{11}C-nicotine (Appelgren et al., 1962). If this observation in mice can be extrapolated to monkeys the main part of the ^{11}C-radioactivity in brain during this time interval must considered to be ^{11}C-nicotine. The uptake of radioactivity to the monkey brain was higher following (+)^{11}C-nicotine than (−)^{11}C-nicotine and radioactivity following (+)^{11}C-nicotine injections declined more rapidly from the brain. Ten min after intravenous injection of (+)^{11}C-nicotine 50% of the maximal radioactivity in frontal cortex had disappeared compared to 30% of the ^{11}C-radioactivity derived from the (−)isomer. This probably indicates that the cerebral blood flow might influences the time course of (+)^{11}C-radioactivity in brain to a greater extent than the (−)^{11}C-radioactivity. Stålhandske (1970) reported a five fold difference in concentration of radioactivity between plasma and brain following ^{14}C-nicotine injections. In this study in monkeys about a two fold difference in ^{11}C-radioactivity between plasma and brain was found for the two isomers. The uptake of ^{11}C-radioactivity was low in areas including muscle and eye. The ^{11}C-radioactivity remained constant up to 60 min in these areas indicating a kinetic for ^{11}C-nicotine different from that in the brain. Maziere et al. (1979) also observed an uptake of ^{11}C-radioactivity to the eye following ^{11}C-nicotine and concluded that the radioactivity might be retained in the retina. The significance of the uptake of ^{11}C-radioactivity to the area including the eye is unclear. Similar to brain the uptake of radioactivity decreased following nicotine pretreatment and increased following mecamylamine administration (data not shown). In contrast to this finding Maziere et al. (1979) observed no reduction in uptake of ^{11}C-nicotine to the brain following intravenous injection of unlabelled nicotine.

To prevent that some of the ^{11}C-nicotine might bind to peripheral nicotinic receptors (for example in blood vessels) a peripheral nicotinic ganglionic blocker was given prior to the injection of ^{11}C-nicotine. At peripheral blockade a lower uptake of (+)^{11}C-radioactivity was observed while the uptake of (−)^{11}C-radioactivity was unchanged. This difference in uptake of ^{11}C-radioactivity indicates that changes in cerebral blood flow influence the uptake of the enantiomers differently. A different pattern in distribution of (−)^{11}C-radioactivity compared to (+)^{11}C-nicotine was observed in brain. Following (−)^{11}C-nicotine a regional difference in distribution of radioactivity was observed while in (+)^{11}C-nicotine injected monkeys the (+)^{11}C-radioactivity was rather similar in different brain areas. Martin et al. (1983) reported following subcutaneous injections of (+) and (−)^{3}H-nicotine to rats a difference in brain content of radioactivity. Following (−)^{3}H-nicotine the radioactivity was two times higher in cortex compared to the cerebellum and medulla oblongata. Following (+)^{3}H-nicotine the brain content of radioactivity was lower and there was less regional differences (Martin et al., 1983). These experiments were performed in the absence of peripheral nicotinic blocker and might therefore illustrate species differences concerning the influence of nicotine on for example cerebral blood flow. Appelgren

et al. (1962) injected intravenously (−)^{14}C-nicotine to cats and studied by autoradiography the distribution of radioactivity in brain. A high uptake of radioactivity was observed in grey matter compared to white with a tendency of higher amount of radioactivity in the diencephalon and medulla oblongata. In order to study nicotinic receptor in brain in vitro receptor autoradiography has been applied to thin tissue section of rat brain (Clarke et al., 1984; London et al., 1984; Clarke et al., 1985; Schwartz, 1986; Härfstrand et al., 1988) as well as human brain (Adem et al., 1988 a, b; Nordberg et al., 1988 b). Quantitative measurements of ^{3}H-nicotine and ^{3}H-ACh labelling indicate a high number of nicotinic receptors in the thalamus, caudate nucleus and different cortical areas (Härfstrand et al., 1988; Adem et al., 1988 a, b). Similar results have also been observed in tissue homogenate binding studies (Larsson and Nordberg, 1985; Larsson et al., 1987; Adem et al., 1987; Adem and Nordberg, 1988). The difference in regional brain content of (+) and (−)^{11}C-radioactivity observed in this study under peripheral nicotinic receptor blockade might represent binding to nicotinic receptors. The time course of specific ^{11}C-nicotine binding reveal a short ligand-receptor interaction with high dissociation rate (approximately 8 min) which is also known from in vitro binding sites with ^{3}H-nicotine (Larsson and Nordberg, 1985). Calculation of receptor binding rate constants will however due to the short drug-receptor interaction time be hampered by great uncertainess (Hartvig et al., 1987). Due to the small size of the monkey brain the anatomical resolution using PET is less precise compared to human brain. We therefore think that these studies motivate a continuation in human brain. A decrease in number of high affinity nicotinic receptors has been reported in postmortem cortical brain tissue from Alzheimer patients (Whitehouse et al., 1986; Nordberg and Winblad, 1986). A concommitant shift in the proportion of high affinity to low affinity nicotinic receptors is also observed in Alzheimer brains (Nordberg et al., 1988 a). PET technique using (−)^{11}C-nicotine might be a promising technique for further understanding of the underlying mechanisms for nicotinic receptors in the brain and their role in nicotine dependence and neurodegenerative disorders such as dementia of the Alzheimer type.

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