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Time and brain region specific up-regulation of low affinity neuronal nicotinic receptors during chronic nicotine administration in mice

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Abstract

We studied the effects of chronic oral nicotine on brain low affinity nicotine binding sites. Mice received nicotine in the drinking water for 4 or 7 weeks. Receptor binding was measured at 24 or 48 h after cessation of nicotine administration with [^{3}H]methyllycaconitine, an antagonist in α7 and α3/α6β2β3* nicotinic receptors in striatum, midbrain, hippocampus and cortex. Chronic nicotine for 4 weeks resulted in a significant increase in the [^{3}H]methyllycaconitine binding in the striatum and cortex, whereas after 7 weeks the increase in binding could be found in the hippocampus but not in the other brain areas studied. For comparison, high affinity nicotine binding sites (mostly α4β2) were measured with [^{3}H]epibatidine after 7-week chronic nicotine treatment. [^{3}H]Epibatidine binding sites were increased in the hippocampus, midbrain and cortex, but not in the striatum. The up-regulation of [^{3}H]methyllycaconitine binding was significant at 24 h but that of [^{3}H]epibatidine binding sites was not observed until at 48 h after cessation of chronic nicotine. These results suggest that up-regulation of low affinity nicotine binding sites does occur during chronic nicotine administration. Furthermore, the low affinity and high affinity binding differ clearly as regards regions and duration suggesting that different nicotinic receptors respond differently to nicotine administration.

Keywords: α7 nicotinic receptor; Neuronal nicotinic receptor; Nicotine; Receptor binding; Methyllycaconitine; Epibatidine

1. Introduction

Pharmacological effects of nicotine are mediated through nicotinic acetylcholine receptors. The multiplicity of nicotinic receptors has been known for a long time since the muscle and ganglionic nicotinic receptors are activated and blocked by different drugs. More recently, molecular biology revealed the multiplicity of nicotinic receptors in the central nervous system. Neuronal nicotinic receptors are composed of α and β subunits. Presently, nine different α subunits (α2–α10) and three different β (β2–β4) subunits have been cloned. Homomeric nicotinic receptors containing α7 subunits and heteromeric nicotinic receptors containing a combination of α4β2 subunits are the most abundant nicotinic receptors in the brain (Nai et al., 2003). The α7 subunits are distributed throughout the brain with a prevalent expression in the cortex, hippocampus and limbic areas. The α7 nicotinic receptors show high affinity for α-bungarotoxin and low affinity for nicotine. They display high relative permeability to calcium, fast desensitization upon acute receptor activation and also rapid recovery from desensitized state (Broide and Leslie, 1999). The α7 nicotinic receptors appear to participate in various cellular events such as neurotransmitter release and neuronal survival and they may have a role in long-term potentiation and other cognitive functions (Levin et al., 1999; Mansvelder and McGehee, 2000).

Up-regulation of nicotine binding is found both in smokers and in experimental animals treated chronically with nicotine (for a review, see Gentry and Lukas, 2002). Indeed, we previously reported that in mice withdrawn from 4- or 7-week chronic oral nicotine treatment the binding of [^{3}H]nicotine in the midbrain and cortex is elevated (Pietilä et al., 1998). Such high affinity nicotine binding is likely

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due to the α4β2 nicotinic receptors, which represent the majority of the high affinity nicotine binding sites in the brain and are widely expressed in central nervous system (Holladay et al., 1997). On the other hand, increased [^{125}I]α-bungarotoxin binding representing the low affinity nicotine binding sites, presumably mostly α7 nicotinic receptors, has also been observed after chronic nicotine administration (Marks et al., 1985, 1986a,b). In general, the increase in [^{125}I]α-bungarotoxin binding after chronic nicotine treatment is regionally more restricted and occurs first after higher nicotine concentrations than the up-regulation of [^{3}H]nicotine binding (Marks et al., 1985; Pauly et al., 1991). Therefore, it has been suggested that the concentrations of nicotine that induce the up-regulation of [^{125}I]α-bungarotoxin binding may be higher than those measured in human smokers (Pauly et al., 1991; Wonnacott et al., 1990). Thus, the low affinity nicotine binding might respond to chronic nicotine differently than the high affinity binding.

The aim of this study was to further clarify the effect of chronic nicotine treatment on the low affinity nicotine binding sites in mouse brain after 4- or 7-week oral nicotine treatment. For receptor binding, we used [^{3}H]methyllycaconitine, which is a new radioligand with nanomolar affinity for α7-type nicotinic receptors (Davies et al., 1999). For comparison, we also examined the effects of chronic nicotine treatment on high affinity nicotine binding sites using [^{3}H]epibatidine as the ligand since it interacts predominantly with α4β2 nicotinic receptors (Marks et al., 1998; Whiteaker et al., 2000).

2. Materials and methods

2.1. Materials

(−)-Nicotine for nicotine drinking solutions was from Fluka BioChemika (Buchs, Switzerland), (−)-nicotine hydrogen tartrate was from Sigma-Aldrich Finland (Helsinki, Finland), [^{3}H]methyllycaconitine (specific activity 26.2–26.5 Ci/mmol) was obtained from Tocris Cookson (Bristol, UK), and [^{3}H]epibatidine (specific activity 48.00 Ci/mmol) was obtained from DuPont NEN (Boston, MA, USA). MultiScreen GF/C glass fibre plates were from Millipore (Billerica, MA, USA).

2.2. Chronic administration of nicotine

Five-week-old male NMRI mice, originally developed in the National Marine Research Institute (Bethesda, Md., USA), were bred locally in the Laboratory Animal Centre of University of Helsinki. The mice (20–31 g) were divided randomly into nicotine-receiving and control mice. All experimental procedures described in this paper were approved by the Committee for Animal Experiments of the University of Helsinki. The mice were housed in groups of five and had free access to mouse chow. The lights were on from 6:00 to 18:00 h, the ambient temperature was held at + 20–22 °C and the relative humidity at 50 ± 10%.

Nicotine was administered chronically in the drinking water (the sole source of fluid) as described by (Pekonen et al., 1993). The concentration of nicotine was gradually increased from 50 μg/ml at 3–4-day intervals to 300 μg/ml over 3 weeks and after this at 7-day intervals to 500 μg/ml over 4 weeks to coax the mice to drink as steadily as possible. In the shorter 4-week treatment period, the nicotine concentration was gradually increased to 350 μg/ml and during the last week the concentration was 350 μg/ml. The control mice drank tap water during the entire treatment. Body weights and fluid intake were recorded once a week. The observed plasma concentrations of nicotine (54 ng/ml) in mice after 7-week oral nicotine exposure are about similar to the nicotine concentrations (10–50 ng/ml) reported in the afternoon in smokers (Benowitz et al., 1982; Pekonen et al., 1993; Russell et al., 1975).

After cessation of nicotine administration, the mice were withdrawn by replacing the nicotine solution with tap water for 24 or 48 h before the receptor binding was done. It has been shown previously that due to their short elimination half-lives neither nicotine nor its metabolite cotinine can be found in the plasmas of the mice after 24 h after withdrawal that could interfere with the receptor binding study (Pekonen et al., 1993; Petersen et al., 1984).

2.3. Preparation of cell membranes for ligand binding assays

After the withdrawal, the mice were killed by decapitation and striatum, hippocampus, midbrain (thalamus and mesencephalon) and cortex were dissected on ice and frozen on dry ice. The brain tissues were weighed (mean weights: striatum 24.0 mg, hippocampus 40.7 mg, midbrain 67.6 mg and cortex 172.3 mg) and stored at − 80 °C until assayed.

To ensure adequate protein concentration, striatal and hippocampal tissue samples of two mice from the same treatment group were pooled when preparing the membrane homogenates. Midbrain and cortical samples were not pooled but were prepared from a single mouse. Brain tissues were homogenized using a motor-driven glass Teflon-homogeniser in 10 volumes of ice-cold sucrose (0.32 M sucrose, 1 mM EDTA, 0.1 mM phenylmethyl sulfonyl fluoride, pH 7.4) and centrifuged at 1000×g, for 10 min at + 4 °C. The supernatant (S_1) was collected and the pellet (P_1) was resuspended in 5-fold volume of fresh ice-cold sucrose and centrifuged at 1000×g for 10 min at + 4 °C. The pellet (P_2) was discarded and the supernatant (S_2) was combined with the supernatant S_1 and centrifuged at 12,000×g for 30 min at + 4 °C. The pellet was washed twice by resuspension in 2.5-fold volume of phosphate

buffer (50 mM potassium phosphate, 1 mM EDTA, 0.1 mM phenylmethyl sulfonyl fluoride, pH 7.4) and centrifugation (12,000×*g*, 30 min, + 4 °C). The final pellet was resuspended in 5-fold volume of phosphate buffer and kept frozen at − 20 °C until the binding experiments were performed. Before the receptor binding assay, the samples were thawed and their protein concentrations were measured by commercial BCA-protein assay kit (Pierce Biotechnology, Rockford, IL, USA).

2.4. [^{3}H]Methyllycaconitine binding to membranes

The binding reaction of [^{3}H]methyllycaconitine was performed in 96-well polystyrene plate in a total volume of 250 μl/well. 150 μl of membrane suspension (45 μg of protein) containing 0.1% (w/v) bovine serum albumin was incubated with 5 nM [^{3}H]methyllycaconitine for 2 h at room temperature. Nicotine (1 mM) was used to determine non-specific binding. Binding reactions were stopped by filtration of samples through wells of Multiscreen glass fibre 96-well filter plate. The wells were pre-soaked with 200 μl of 0.5% polyethylene imine (v/v) overnight at + 4 °C after which the wells were aspirated on the vacuum manifold and washed three times with the assay buffer. After filtration of the samples, the wells were washed 6 times with ice-cold assay buffer (200 μl/well). The plastic underdrain was carefully removed and the plate was dried. Scintillation cocktail (30 μl, OptiPhase HiSafe 3, Wallac, Turku, Finland) was added to each well and the plate was left to equilibrate at least for 6 h before counting in a beta counter (MicroBeta® TriLux, Wallac, Turku, Finland). All the samples from different treatment groups of one brain area were measured in the same experiment.

2.5. [^{3}H]Epibatidine binding to membranes

Membrane samples (containing 45 μg of protein) were incubated in Multiscreen 96-well glass fibre plates with 1 nM [^{3}H]epibatidine for 2.5 h in a total volume of 250 μl at room temperature. Each well of the plate was pre-soaked with 200 μl of 0.15% polyethylene imine (v/v) and washed before incubation as described above. Nicotine (1 mM) was used to determine non-specific binding. After incubation, the filter plate was subsequently aspirated on the vacuum manifold and washed 4 times with 200 μl/well of ice-cold assay buffer and the plate was prepared for the counting as presented in [^{3}H]methyllycaconitine binding. All the samples from different treatment groups of one brain area were measured in the same experiment.

2.6. Statistics

Values are the mean ± S.E.M. of the specific binding. The variances in the control and nicotine treated groups were tested with a two-sample *F*-test. The statistical analyses were carried out by Student's *t*-test.

3. Results

3.1. [^{3}H]Methyllycaconitine binding

The binding of [^{3}H]methyllycaconitine to membrane homogenates prepared from striatum of control mice was about 30 fmol/mg protein, to membranes from midbrain 45 fmol/mg protein and to that of hippocampus 70 fmol/mg protein and to cortical membranes about 35 fmol/mg protein.

In mice given nicotine for 4 weeks, the striatal [^{3}H]methyllycaconitine binding was significantly increased after 24 h withdrawal (by 83%, $P<0.05$), and a tendency towards up-regulation was also found after 48 h, but it was not statistically significant (Fig. 1). The 4-week nicotine administration did not affect the specific [^{3}H]methyllycaconitine binding to the midbrain or hippocampal membranes (Fig. 1). The 4-week nicotine exposure significantly increased the cortical [^{3}H]methyllycaconitine binding in mice withdrawn for 24 h (by 67%, $P<0.01$) or 48 h (by 55%, $P<0.01$) (Fig. 1).

The 7-week nicotine administration in the drinking water did not affect the binding of [^{3}H]methyllycaconitine to membranes prepared from striatum (Fig. 2A). The 7-week nicotine treatment significantly increased the hippocampal [^{3}H]methyllycaconitine binding in mice withdrawn for 24 h (by 64%, $P<0.05$) or 48 h (56%, $P<0.05$) (Fig. 2A). The 7-week nicotine administration did not affect [^{3}H]methyllycaconitine binding to the midbrain or cortical membranes (Fig. 2).

3.2. [^{3}H]Epibatidine binding

The binding of [^{3}H]epibatidine to membrane homogenates prepared from striatum from control mice was about 200 fmol/mg protein, to membranes from midbrain about 300 fmol/mg protein and to that of hippocampus 80 fmol/

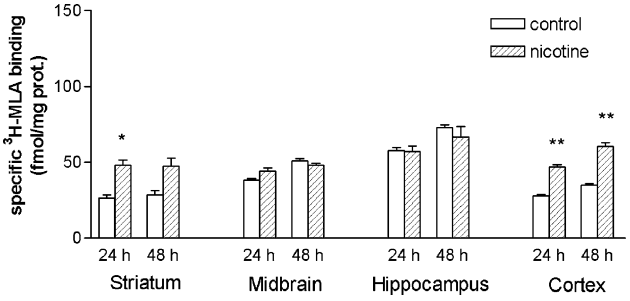

Fig. 1. Effects of chronic oral nicotine on the number of [^{3}H]methyllycaconitine ([^{3}H]MLA) binding sites in striatal, midbrain, hippocampal and cortical membranes of mice withdrawn from nicotine for 24 or 48 h. Nicotine was administered to mice for 4 weeks. Striata or hippocampi from two mice and midbrain or cortex from one mouse were used to prepare the membrane preparation, which was incubated with 5 nM [^{3}H]MLA. Data are given as fmol/mg protein. Means ± S.E.M. from 4–5 (striatum), 5 (hippocampus), 7–10 (midbrain) and 9 (cortex) measurements. *$P<0.05$, **$P<0.01$.

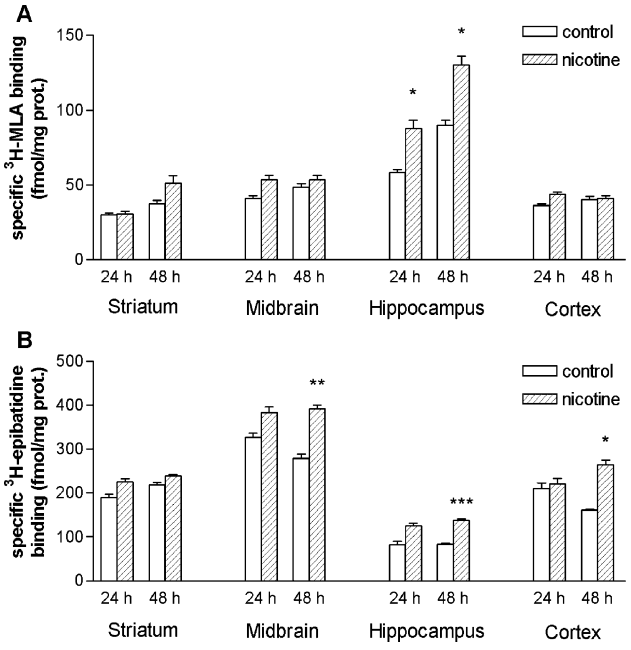

Fig. 2. Effects of chronic oral nicotine on the number of [^{3}H]methyllycaconitine ([^{3}H]MLA) (A) and [^{3}H]epibatidine (B) binding sites in striatal, midbrain, hippocampal and cortical membranes of mice withdrawn from nicotine for 24 or 48 h. Nicotine was administered to mice for 7 weeks. Striata or hippocampi from two mice and midbrain or cortex from one mouse were used to prepare the membrane preparation, which was incubated with 5 nM [^{3}H]MLA or 1 nM [^{3}H]epibatidine. Data are given as fmol/mg protein. Means±S.E.M. from 4–5 (striatum), 4–7 (hippocampus), 4–9 (midbrain) and 4–10 (cortex) measurements. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

mg protein and to cortical membranes 185 fmol/mg protein.

The binding of [^{3}H]epibatidine to striatal membranes was not affected either at 24 or at 48 h after cessation of the 7-week nicotine administration in the drinking water (Fig. 2B). The 7-week nicotine administration in the drinking water followed by 24-h withdrawal period did not significantly affect the [^{3}H]epibatidine binding in the midbrain (Fig. 2B), but a significant increase in the [^{3}H]epibatidine binding was found in mice withdrawn for 48 h (40%, $P<0.01$).

The 7-week nicotine exposure significantly increased the hippocampal [^{3}H]epibatidine binding in mice withdrawn for 48 h (by 66%, $P<0.001$), but the increase found after 24 h (52%) of withdrawal was not statistically significant (Fig. 2B). The binding of [^{3}H]epibatidine to cortical membranes was not affected after 24 h of withdrawal from 7-week nicotine administration in the drinking water but withdrawal for 48 h resulted to a significant increase in the [^{3}H]epibatidine binding (64%, $P<0.05$).

4. Discussion

In the present study, we show that the low affinity and the high affinity nicotine binding sites in various brain regions are increased in mice chronically treated with nicotine, as estimated by the [^{3}H]methyllycaconitine and [^{3}H]epibatidine binding, respectively.

When the low affinity binding sites were labelled with [^{3}H]methyllycaconitine, a significant up-regulation was observed in the striatum and cortical areas after 4-week, but not after 7-week nicotine treatment. In the hippocampus, significant up-regulation was observed only after 7-week chronic nicotine administration and no up-regulation was found in the midbrain either at 4 or 7 weeks. The largest up-regulation of the low affinity nicotine binding sites was found in the striatum (83% increase) followed by the hippocampus and cortical areas (about 55–65% increase in both). In agreement with our data, Marks et al. (1985, 1986a,b) found that constant intravenous infusion of nicotine to mice for 8–12 days increases low affinity binding as measured by [^{125}I]α-bungarotoxin in the cortical and hippocampal areas, but not in the midbrain. In contrast to our observation, they did not find up-regulation of [^{125}I]α-bungarotoxin binding in the mouse striatum (Marks et al., 1985, 1986a). It must be noted that in their studies high doses of nicotine (up to 7 mg/kg/h) were required to induce up-regulation of [^{125}I]α-bungarotoxin binding during a relatively short period of administration. Comparable to the 4-week treatment in this study, Sparks and Pauly (1999) have shown that oral administration of nicotine for 30 days (200 μg/ml, sweetened with saccharin) to C57B1/6 mice increases the density of [^{125}I]α-bungarotoxin binding, although rather slightly, in various brain areas (Sparks and Pauly, 1999). [^{3}H]Methyllycaconitine has been used as an alternative to [^{125}I]α-BGT for labelling α7 nicotinic receptors. With nanomolar concentrations, [^{3}H]methyllycaconitine labels in rodent brain a single population of sites that closely parallel [^{125}I]α-bungarotoxin binding sites (Davies et al., 1999; Whiteaker et al., 1999).

The high affinity binding sites for nicotine were labelled with [^{3}H]epibatidine. We found that after 7-week oral nicotine treatment the high affinity binding sites were significantly increased in the hippocampus (by 66%), in the midbrain (by 40%) and in the cortex (by 64%) when measured at 48 h but not 24 h after cessation of oral nicotine. No statistically significant increase in the [^{3}H]epibatidine binding was found in the striatum. Our results are in agreement with several studies showing an up-regulation of high affinity nicotine binding sites in the mouse brain (Collins et al., 1989; Marks et al., 1985, 1992; Pauly et al., 1991; Sparks and Pauly, 1999), and also consistent with previous studies where no up-regulation of high affinity nicotine binding sites occurred in the mouse striatum (Marks et al., 1983, 1993).

Among the four brain areas studied, the hippocampus and the cortex were the only ones in which both the low affinity and the high affinity binding sites were increased after chronic nicotine treatment. The magnitudes of up-regulation of the low affinity versus the high affinity binding sites were equal in the hippocampus and the cortex (55–65% increase). Furthermore, no up-regulation of low affinity but some up-

regulation of high affinity binding was found in the striatum, and vice versa in the midbrain only low affinity binding was up-regulated. Thus, our results are in contrast to those of Pauly et al. (1991), Sparks and Pauly (1999) and Mugnaini et al. (2002), who found that nicotine-induced increase in the density of low affinity nicotine binding sites is regionally more restricted, and not as great in magnitude as the increase in the high affinity nicotine binding sites (Mugnaini et al., 2002; Pauly et al., 1991; Sparks and Pauly, 1999). The up to 7-week long duration of nicotine treatment in our study may be responsible for the up-regulation of low affinity sites we found. Indeed, in some of the human studies, more up-regulation of nicotinic receptors has been reported compared to rodents, and this difference was suggested to be due to a longer exposure to nicotine in humans than in rodent studies (Gentry and Lukas, 2002).

Oral administration of nicotine in drinking water in mice produces nicotine concentrations in plasma which mimic those observed in smokers (Benowitz et al., 1982; Pekonen et al., 1993; Russell et al., 1975). This is because mice receive nicotine mainly during their active period of the day in a manner, which produces peaks in blood nicotine concentrations similarly to smoking. It has earlier been shown that the plasma concentrations of nicotine are about similar both after 4 (35 ± 10 ng/ml) and 7 weeks (50 ± 20 ng/ml) of oral nicotine administration (Pekonen et al., 1993), suggesting that the nicotine induced increase in [^{3}H]methyllycaconitine binding in hippocampus found in this study is a result of long exposure time rather than high concentration of nicotine. Our data suggest that periods of intermittent nicotine administration for as long as 30 or 50 days are needed to show the effects of nicotine on low affinity nicotine binding sites.

Interestingly, in those areas where up-regulation occurred, [^{3}H]methyllycaconitine binding was increased already after 24 h of withdrawal, whereas a significant increase in [^{3}H]epibatidine binding was not seen until after 48 h of abstinence from oral nicotine treatment. As was the case with [^{3}H]epibatidine binding in the present study in our previous study a statistically significant increase in [^{3}H]nicotine binding in mouse midbrain was found only after 48 h after cessation from chronic oral nicotine administration (Pietilä et al., 1998). Presumably high affinity nicotinic receptors are up-regulated during chronic nicotine exposure and few days after the treatment. However, only part of the up-regulated receptors may be located on the cell membrane and, therefore, the up-regulation can not be seen with the receptor binding measured from membrane samples. It can be hypothesized that the termination of a long nicotine treatment leads to a shift of receptors from intracellular pools to the plasma membrane in order to maintain the response to the nicotinic agonist, and this process may differ among different receptor types. Thus, the kinetics of high affinity nicotinic receptors may be slower than that of low affinity nicotinic receptors known to fast desensitize and recover from desensitization.

Certain of the behavioural effects of nicotine such as the modulation of locomotor activity, reinforcement and addiction are mediated mainly through mesostriatal dopamine pathway. Nicotine can regulate release of dopamine by activation of nicotinic receptors within the striatum, ventral tegmental area and substantia nigra (Picciotto et al., 1998; Wonnacott et al., 2000; Zhou et al., 2001). There are multiple nicotinic receptor subtypes present in these areas, including α7, α4β2 and most likely α6 subunits containing receptors (for a review, see Quik and Kulak, 2002). In the present study, 4-week chronic nicotine treatment resulted to an increase in [^{3}H]methyllycaconitine binding in striatum. Mogg and co-workers showed that methyllycaconitine is a potent antagonist of (−)-conotoxin-MII-sensitive nicotinic receptors in rat striatum, suggesting that, in addition to α7 nicotinic receptors, methyllycaconitine may interact with α3/α6β2β3* nicotinic receptors on dopamine neurons (Mogg et al., 2002). In addition, a recent study suggested that nicotinic receptors mediating dopamine release in the mouse brain contain mainly α4, α5 and α6 subunits in combination with β2 and β3 subunits (Salminen et al., 2004). Furthermore, Nguyen et al. showed that α6/α3β2-like binding was increased by 260% of control in nucleus accumbens of rats treated with nicotine for 2 weeks (Nguyen et al., 2003). Therefore, it is possible that the observed up-regulation of low affinity nicotinic binding sites in striatum includes α3/α6 subunits containing nicotinic receptors in addition to α7 nicotinic receptors. It is possible that the up-regulated receptors in striatum are still functional and mediate the effects of nicotine. Functional up-regulation is a novel theory (for a review see Buisson and Bertrand, 2002) in contrast to the dominant hypothesis of nicotinic receptor up-regulation in which receptor inactivation has been suggested to be the mechanism leading to receptor up-regulation. In agreement with this hypothesis, we have previously shown that chronic oral nicotine treatment continues to activate nicotinic receptors regulating the release of dopamine in nucleus accumbens (Gaddnas et al., 2001). Recently, Nguyen et al. also showed that high affinity nicotine binding sites labelled with [^{3}H]epibatidine are up-regulated but functional after 2-week nicotine treatment in rat brain (Nguyen et al., 2004). Surprisingly, in the present study, we did not find up-regulation of [^{3}H]methyllycaconitine binding after 7-week nicotine exposure in striatum. Thus, if up-regulated receptors remain functional, the lack of receptor up-regulation may reflect the development of tolerance to the effects of nicotine. Indeed, we previously found that tolerance to nicotine's locomotor depressant effect is developed only after 7 weeks of nicotine use and no tolerance of locomotor depression was found after 4 weeks of chronic oral treatment (Pietilä et al., 1998).

Nicotine has been shown to improve cognitive function including working memory and attention in humans and in experimental animals (Foulds et al., 1996; Levin and Simon, 1998). Hippocampal and prefrontal cortical areas have been

implicated in the cognitive effects of nicotine. Both of these areas are known to express moderate to high levels of α7 nicotinic receptors (Seguela et al., 1993). In the present study, we found that up-regulation of [^{3}H]methyllycaconitine binding appeared earlier in the cortex than in the hippocampus. However, the up-regulation of low affinity binding in the cortex was no longer observed simultaneously with that in the striatum after 7 weeks of nicotine exposure. Our results are in agreement with a recent study of Teaktong et al. where the expression α7 nicotinic receptor subunits was found to be increased in hippocampus but not in the entorhinal cortex of human smokers (Teaktong et al., 2004). Thus, it is possible that the low affinity nicotinic receptors in the hippocampus and in the cortex are regulated differentially and that the receptors in the cortical areas are more readily up-regulated but on the other hand, they also develop rapidly tolerance to the up-regulating effect of nicotine. The finding that the α7 nicotinic receptors are located in different types of neurons in the hippocampus and in the cortex (Alkondon and Albuquerque, 2004) and the observation that dorsal hippocampal nicotinic receptors are relatively insensitive to nicotine (Vizi and Kiss, 1998) may partly explain these differences.

In conclusion, the present study shows that the low affinity nicotine binding sites, mainly α7 nicotinic receptors, are up-regulated during chronic nicotine exposure. The manner in which animals receive nicotine is of importance and we suggest that when administered in the drinking water the nicotine concentrations in the plasma mimic those of smokers. The duration of nicotine treatment is also important, and our results suggest that the effect of nicotine on low affinity binding sites differs after 4-week and after 7-week administration. In addition, our results show that the regulation of low affinity nicotinic receptors during nicotine exposure may vary in different brain areas, which might result from different responses of different types of neurons.

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