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Further evidence for the functional role of nonsynaptic nicotinic acetylcholine receptors

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Abstract

The function of nicotinic acetylcholine receptors in the main central systems has been documented in the past decade. These studies focused mostly on the synaptic functions, although acetylcholine is released dominantly into the extrasynaptic space and the majority of nicotinic acetylcholine receptors on remote neurons are found on extrasynaptic membranes. Here, we show further evidence for the role of nonsynaptic nicotinic functions in the cognitive and the reward system. Dendrites of γ-amino-*n*-butyric acid (GABA)-containing interneurons of the hippocampus are densely equipped with nicotinic acetylcholine receptors. These cells play an important role in memory processing. We analysed the effects of nicotinic acetylcholine receptor stimulation on the Ca^{2+} dynamics of interneurons in different dendritic compartments. We also investigated the role of nicotinic receptors in the nucleus accumbens where nicotine stimulated vesicular dopamine release via activation of receptors located on varicosities. Nicotine produced comparable effects with 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) on dopamine release. These examples demonstrate that nonsynaptic nicotinic acetylcholine receptors can effectively influence activity pattern of neural networks in key structures of central systems.

Keywords: Nicotine; Nonsynaptic; Transmitter release; Two-photon imaging; Ca^{2+}

1. Introduction

Discoveries of postsynaptic nicotinic acetylcholine receptors have forced reconsideration of the previous widely held view that all central nicotinic acetylcholine receptors are presynaptic. Activation of nicotinic acetylcholine receptors alters a variety of cognitive and behavioural functions within the diffuse terminal field of central cholinergic projections. Nicotinic acetylcholine receptors are integral membrane proteins composed of five subunits selected from the set of α2–α9 and β2–β4 (Lindstrom, 1995). The multiplicity of subunits may have clinical relevance if we will be able to selectively modulate different nicotinic function in various parts of the brain, and separate the beneficial effects from the unwanted side effects of nicotine administration using subtype-specific ligands. In the human brain the high level of nicotine binding (Perry et al., 1993; Rubboli et al., 1994) should provide the structural basis for the role of nicotinic receptors in learning and memory. The addictive power of nicotine during smoking may be linked to the known potential of nicotinic acetylcholine receptors to improve synaptic plasticity in regions of the brain reward system including the ventral tegmental area and the nucleus accumbens (Dani et al., 2001).

Interneurons of the hippocampus exhibit radioactive α-bungarotoxin labelling and γ-amino-*n*-butyric acid (GABA) immunoreactivity (Adams et al., 2001). Nearly all of the interneurons in the stratum radiatum of the hippocampal CA1 region show dense radioactive α-bungarotoxin binding (Frazier et al., 1998b). On these interneurons α3, α4, α5, α7, β2, β3 and β4 subunits can be found (Sudweeks and

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Yakel, 2000, Hill et al., 1993; Kawai et al., 2002). Interestingly, postsynaptic α7 nicotinic acetylcholine receptors are found in activity-dependent clusters at GABAergic synapses (Kawai et al., 2002). There are several observations demonstrating the existence of nicotinic acetylcholine receptors at nonsynaptic sites (Fabian-Fine et al., 2001; Hill et al., 1993; also Horch and Sargent, 1995; Williams et al., 1998; Ullian and Sargent, 1995).

Hippocampal inhibitory interneurons, especially stratum radiatum cells, can be excited by activation of nicotinic acetylcholine receptors if nicotinic currents were isolated by a synaptic cocktail (Jones and Yakel, 1997; Frazier et al., 1998a; McQuiston and Madison, 1999; Alkondon et al., 1997). These interneurons are able to inhibit the output of a large number of principal excitatory neurons providing possibility of a nicotine-induced disinhibition. The existence of both α7 and non-α7 nicotinic acetylcholine receptors (Alkondon and Albuquerque, 2001; Sudweeks and Yakel, 2000; Shao and Yakel, 2000; McQuiston and Madison, 1999; Frazier et al., 1998b; Jones and Yakel, 1997) were shown. Functional nicotinic acetylcholine receptors were further shown in stratum radiatum interneurons by current responses to carbachol uncaging (Khiroug et al., 2003). In 15–20% of stratum radiatum interneurons fast synaptic transmission is mediated via nicotinic receptors (Alkondon et al., 1998; Frazier et al., 1998a). The net effect of nicotinic stimulation in stratum radiatum interneurons is an inhibition on the pyramidal neurons (Buhler and Dunwiddie, 2002). However, disinhibition may appear in pyramidal neurons when the GABAergic input exhibits high spontaneous activity (Ji and Dani, 2000). More than 85% of cholinergic varicosities are nonsynaptic in cortical areas and in the hippocampus (Descarries and Mechawar, 2000). Therefore, the released acetylcholine diffuses dominantly in the extrasynaptic space to reach its target receptor, which should be on the extrasynaptic surface of the remote neurons.

It has been suggested that addiction to nicotine is also linked to an increase in dopamine release via activation of the β2 subunit-containing presynaptic nicotinic acetylcholine receptors in mesolimbic dopamine neurons (Corrigall et al., 1992; Rasmussen and Swedberg, 1998). An important anatomical substrate of brain reward mechanisms is the nucleus accumbens. Administration of abused drugs activates the reward pathways and increases dopamine concentrations in the synaptic cleft and the extracellular space (Di Chiara and Imperato, 1988; Volkow et al., 1997). It is generally accepted that dopamine released in this structure plays a central role in reward processes thus it is heavily involved also in addiction mechanisms (Wise and Bozarth, 1987; Koob, 1992). Anatomically, the nucleus accumbens is not a homogenous structure since it can be divided into two markedly different parts, the shell and the core (Alheid and Heimer, 1988). Recent data clearly show that the two subregions are functionally different (Sellings and Clarke, 2003). The medial part of the nucleus accumbens, the shell, participates in the development of reward while the lateral part, the core, is the mediator of behavioural activation. Regarding the addictive potential of nicotine, it is reasonable to assume that the nucleus accumbens is also involved in the addiction to smoking. Previously it has been shown that intravenous administration of nicotine induced an increase of dopamine release in the shell but not in the core (Pontieri et al., 1996). In the present study, we provide evidence that the phenomenon can be observed also in anaesthetized rat, where the possible interference of behavioural factors can be excluded. In addition, the subcutaneous injection of nicotine, in contrast to intravenous administration, better resembles to smoking in that nicotine concentration develops gradually.

Blood level of nicotine in smokers may reach the firing threshold in ventral tegmental area neurons leading to dopamine release in the nucleus accumbens. The nicotine concentration in the blood of smokers is ranging between 250 and 500 nM for about 10 min after smoking a cigarette (Henningfield et al., 1993). During the first minute of smoking a cigarette, blood level of nicotine goes up to 250 nM (Henningfield et al., 1993). The distribution half-life of 8 min and the elimination half-life of 2 h together result in activation of nicotinic acetylcholine receptors then the prolonged low level of nicotine will favour desensitisation (Pidoplichko et al., 2004). The long half-life of nicotine and slow recovery from desensitisation may explain why smokers report that the first cigarette is the most pleasurable of the day (Dani and Heinemann, 1996). Because a relatively low concentration of nicotine can be reached during smoking, the most likely effect of this drug is on the receptors of high affinity. Synaptic receptors usually respond to extreme (in the order of mM) high concentrations of transmitters, while receptors located on the extrasynaptic surface of the neuron participate in nonsynaptic transmission, and are sensitive to much lower concentrations making them the most likely targets of medicines (Vizi, 2000). Therefore, it seems reasonable to assume that the main mechanism through which nicotine achieves its central effects is the activation and/or desensitisation of nonsynaptic receptors.

Here we show evidence for various functions of nonsynaptic nicotinic acetylcholine receptors in the hippocampus, where the nicotinic modulation of the GABAergic neurons has been investigated, and in the nucleus accumbens, an important structure of the reward system, where the effects of nicotine on dopamine release from dopaminergic terminals have been studied.

2. Materials and methods

2.1. Electrophysiology

Transverse slices (300 μm) containing the hippocampus from 16- to 19-day-old Wistar rats were dissected as described previously (Zelles et al., 2001). Interneurons in the stratum radiatum of the CA1 hippocampal subfield were

visualized under video infrared-DIC. For current clamp recordings electrodes were filled with 125 mM K-gluconate, 20 mM KCl, 10 mM HEPES, 10 mM Di-Tris-salt phosphocreatine, 0.3 mM Na-GTP, 4 mM Mg-ATP, 10 mM NaCl and 112 μM Oregon Green BAPTA-1. Synaptic stimulation was performed with a unipolar glass microelectrode filled with artificial cerebrospinal fluid (ACSF). Stimulus intensity was always maintained at a minimum to reach the threshold of synaptic responses. Data acquisition and analysis were performed using an Axopatch-1D amplifier and pClamp8 (Axon Instruments, USA).

2.2. Two-photon imaging

Imaging was performed using a custom-made two-photon laser scanning system consisting of a modified confocal microscope (Olympus Fluoview, Germany) and a titanium–sapphire laser (Millenia/Tsunami, SpectraPhysics, USA) providing 100 fs pulses at 80 MHz at a wavelength of 820 nm. High-time-resolution fluorescence measurements were obtained in line-scan mode (6.144 ms temporal resolution) after zooming onto a dendritic section. Data recording was started 20–25 min following break-in. At the end of each experiment, a series of images across the depth of the volume encompassing the imaged neuron was taken. Image data were analysed off-line using a custom-made program written in Matlab. Fluorescence traces are expressed as relative fluorescence changes [$\Delta F/F=(F-F_0)/F_0$], where F_0 is the background-corrected pre-stimulus fluorescence. Drugs were administered using a puffer pipette placed 50–100 m from the imaged cell body and a pressure ejection device (DAD-12, ALA Scientific Instruments, NY, USA).

2.3. [^{3}H]dopamine release experiments

Male Wistar rats weighing 120–150 g were anaesthetised and decapitated. One group of rats were intraperitoneally injected daily with reserpine (5 mg/kg) for 2 days before the experiment. Brains were removed and rinsed in Krebs solution. Slices of the microdissected tissue samples from the nucleus accumbens were loaded with [7,8 ^{3}H]dopamine (Amersham International Plc, UK) for 40 min at 37 °C in Krebs solution and aerated with 95% O_2 and 5% CO_2. Slices were then superfused with Krebs solution at 37 °C at a rate of 1 ml/min. After the pre-perfusion period (60 min) 3-min samples of the effluent were collected and assayed for radioactivity by liquid scintillation spectroscopy (Packard Tri-Carb 1900TR; USA). The tissues were electrically stimulated (2 Hz for 2 min). Radioactivity was expressed as disintegrations per minute per gram of tissue (Becquerel/g) or as fractional release (FR), i.e. as a percentage of the total radioactivity in the tissue at the time of sample collection. The effects of drugs were determined as the ratio of FR values in the presence of the drug and the FR values before the drug reached the slices (FRR_2/FRR_1).

2.4. Microdialysis experiments

Male Wistar rats (280–350 g) were anaesthetised with urethane (1300 mg/kg). The animals were placed in a stereotaxic frame and a homemade I-shaped dialysis probe (membrane: Spectrapor, permeability: 6000 D MW, 0.2 mm outer diameter, 2 mm active length) was implanted into the core or shell of the nucleus accumbens. The probe was perfused with a modified Ringer's solution at a rate of 2.0 μl/min (CMA 100 microdialysis pump, Carnegie, Sweden). Following the stabilization of dopamine release, after a 60-min equilibrium period, 15-min samples were collected. After collection of four basal samples, the animals received a subcutaneous injection of nicotine (0.8 mg/kg) and the effect was studied in the next 60 min. The placement of the probe was verified post mortem by stereomicroscopic examination. Animal procedures complied with the NIH guidelines. The dopamine content of samples was determined by High Pressure Liquid Chromatography consisting of a Shimadzu LC-10ADVP pump (Kyoto, Japan), a Coulochem II electrochemical detector (ESA, USA) and an electrically actuated injection valve with a 20 μl loop (VALCO, Switzerland). Separation was achieved on a reversed-phase column (Supelco LC-18-DB, Bellefonte, USA). Chromatograms were collected and analysed by the MAXIMA 820 chromatography software. The detection limit of the assay was 0.2–0.3 pg/sample (on column).

2.5. Statistical analysis

Results were expressed as means±S.E.M. and analysed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test. Statistical significance was set at $P<0.05$. For the microdialysis experiments the average concentration of three samples before treatment was taken as 100% and all values were expressed relative to this control.

2.6. Materials

(−)-Nicotine hydrogen tartarate and (±)-3,4-methylenedioxymethamphetamine hydrochloride (MDMA) were purchased from Sigma-Aldrich, Budapest, Hungary. Reserpine was obtained from Gedeon Richter, Budapest, Hungary. All chemicals used were of analytical grade.

3. Results

3.1. Functional nonsynaptic nicotinic receptors in dendrites of interneurons

Ca^{2+} dynamics in dendrites of hippocampal CA1 interneurons was revealed by two-photon laser scanning imaging of Oregon Green BAPTA-1-filled cells in the stratum radiatum. The rapid pressure application of acetylcholine

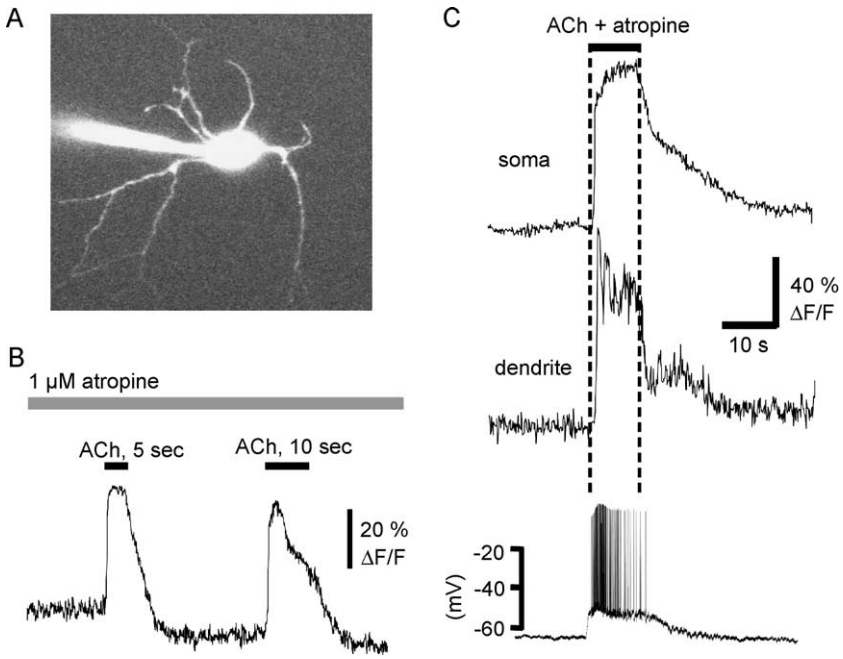

Fig. 1. CA1 stratum radiatum interneurons of the hippocampus express functional nAChRs. (A) Stratum radiatum interneurons in the CA1 region of the hippocampus were filled with Oregon Green BAPTA-1 (112 μM) via a patch pipette. Collapsed two-photon image stack of an Oregon Green BAPTA-1-filled interneuron. (B) Application of acetylcholine (ACh) in 1 mM concentration produced reproducible increases in the intracellular Ca^{2+} concentration. (C) Ca^{2+} responses appear both in the somatic and the dendritic compartments.

produced reproducible increases in the intracellular Ca^{2+} level (Fig. 1B). Large Ca^{2+} transients were observed in neurons, which fired many action potentials in the presence of the agonists (10 cells out of 24; see example traces in Fig. 1C). Atropine (1 μM) was present in the bath throughout the experiment in order to exclude the muscarinic acetylcholine

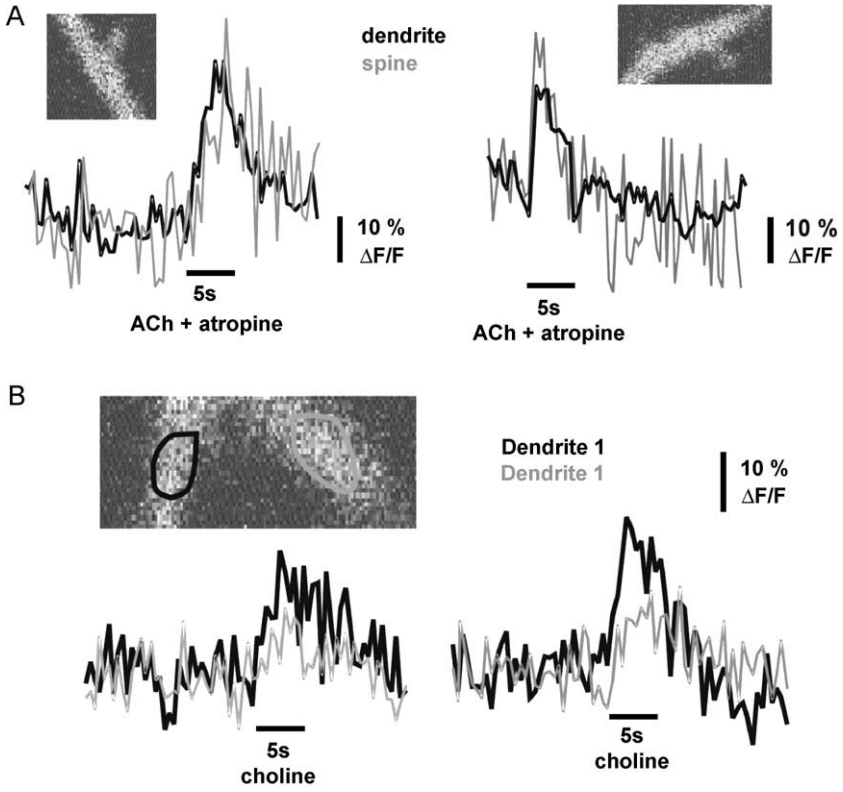

Fig. 2. Nicotinic receptor stimulation-evoked Ca^{2+} signalling in different regions of CA1 interneurons. (A) Example traces showing homogenous effects of nicotinic acetylcholine receptor stimulation in spines and dendrites. (B) In bifurcations of dendrites responses were maintained in both dendrites.

receptor-mediated effects. Following the pressure ejection, the concentration of the nicotinic agonists diluted out in a large extent, especially in case of dendrites at 20–50 μm depths. Nicotinic agonists could produce rises in the intracellular Ca^{2+} level without firing of action potentials (Fig. 2) suggesting a direct dendritic site of action. Therefore, it is reasonable to assume that most of the receptors that mediated this effect were nonsynaptic high-affinity nicotinic acetylcholine receptors of the interneurons. Ca^{2+} responses in different regions of the cells were largely intact: both the somatic and the dendritic compartment responded with comparable amplitude (Fig. 1C). In order to map the region specificity of the responses, we compared nicotinic acetylcholine receptor stimulation-evoked Ca^{2+} transients at dendritic compartments in the absence of action potentials. A fraction of interneurons contained a few dendritic spines, which responded to acetylcholine in a similar fashion to their adjacent dendrites (Fig. 2A). Different dendrites near the bifurcation still showed Ca^{2+} responses to rapid nicotinic stimulation (Fig. 2B). Choline activates α7 receptors (producing whole cell currents) in hippocampal neurons with an EC_{50} of 1.6 mM (Alkondon et al., 1997; Mike et al., 2000). In our study choline acted with similar potency as acetylcholine to produce intracellular Ca^{2+} accumulation in dendrites of hippocampal interneurons (Fig. 2B) suggesting that the α7 subunit of nicotinic acetylcholine receptors was involved in the acetylcholine-mediated actions.

3.2. Effect of nicotinic receptor stimulation depends on the firing properties

Short trains of backpropagating action potentials were evoked by somatic current injection (90–170 pA, 15 ms, 5 action potentials, 29 Hz) to induce dendritic Ca^{2+} responses (Fig. 3A). Pressure application of choline sensitised the Ca^{2+} response to backpropagating spikes: the same current injection produced 8–10 action potentials (n=3 cells, Fig. 3A). Choline was able to make cells fire action potentials (5–6 Hz) for longer time periods. The evoked 29 Hz trains of action potentials failed to induce Ca^{2+} elevations under these circumstances (Fig. 3B). This indicates that the nicotinic acetylcholine receptor stimulation is capable to facilitate or depress the response to a high frequency train depending on the spontaneous firing.

3.3. Nicotinic facilitation of synaptic responses in dendrites of interneurons

In stratum radiatum interneurons synaptic stimulation causes dendritic Ca^{2+} responses, which restrict to 12–14-μm-long compartments along the dendrite (Rozsa et al., 2004). Coincident stimulation of nicotinic acetylcholine receptors enhanced the synaptically evoked Ca^{2+} transients in these compartments (Fig. 4A). The facilitation, induced by the pressure ejected nicotinic agonist, disappeared after washing out the drug (Fig. 4B). Interestingly, the length of

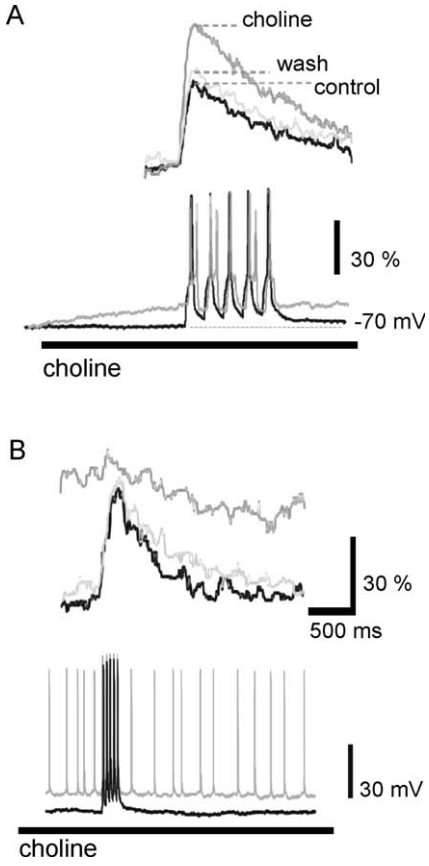

Fig. 3. Plastic properties of nAChRs located on dendrites of hippocampal interneurons. (A) Choline puff application and coincident backpropagating action potentials (bAPs) produced larger Ca^{2+} accumulation in response to bAPs. (B) Simultaneous firing of the cell prevented the generation of bAP-evoked Ca^{2+} transients.

the compartment showed a threshold-dependent change when the net Ca^{2+} response within the compartment substantially increased. The nicotinic facilitation of the evoked synaptic response rather focused on the central region of the compartment (Fig. 4C).

3.4. Effect of nicotine on dopamine release in vitro

After the slices from the isolated tissue punches from the nucleus accumbens had been loaded with [^{3}H]dopamine, the resting release of radioactivity was 9460±689 Bq/g (FR=0.73±0.04%; n=32), and was maintained throughout the superfusion experiment. The two subregions were not separated in these experiments. MDMA and nicotine significantly increased the resting release of dopamine (Fig. 5A,B). The release of dopamine produced by MDMA and nicotine were compared while nicotine induced a large dopamine release within 30 s (Fig. 5A), MDMA perfusion had to be maintained for several minutes to produce a similar amount of release. In order to determine the release mechanism of nicotine-induced dopamine release we used reserpine pretreatment to exclude the effect through the transmitter containing synaptic vesicles. Electrical stimula-

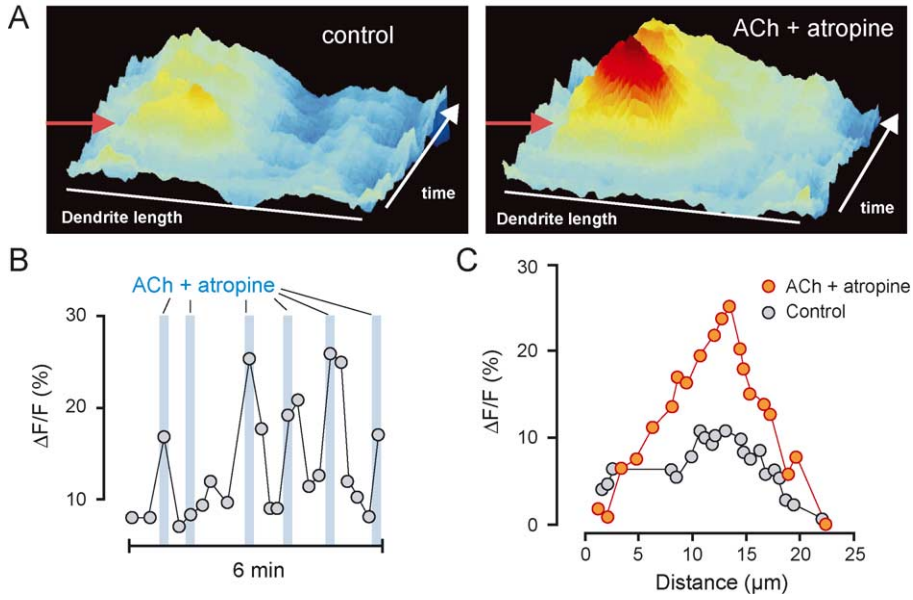

Fig. 4. Stimulation of nicotinic acetylcholine receptors facilitates synaptic stimulation-evoked Ca^{2+} accumulation in dendrites of interneurons. (A) Three-dimensional demonstration of synaptic stimulation-evoked Ca^{2+} responses in a 14-μm-long dendritic segment of a CA1 interneuron. Red arrows denote the time of synaptic stimulation. The original materials were line scan images going parallel with the dendritic segment. (B) Amplitudes of the evoked Ca^{2+} responses ($\Delta F/F$ values) are plotted against time. Note the rapid wash out of the nicotinic enhancement. (C) During the nicotinic receptor stimulation (acetylcholine, 1 mM+atropine 1 μM) synaptic Ca^{2+} responses were greatly facilitated within the borders of the synaptic Ca^{2+} compartment.

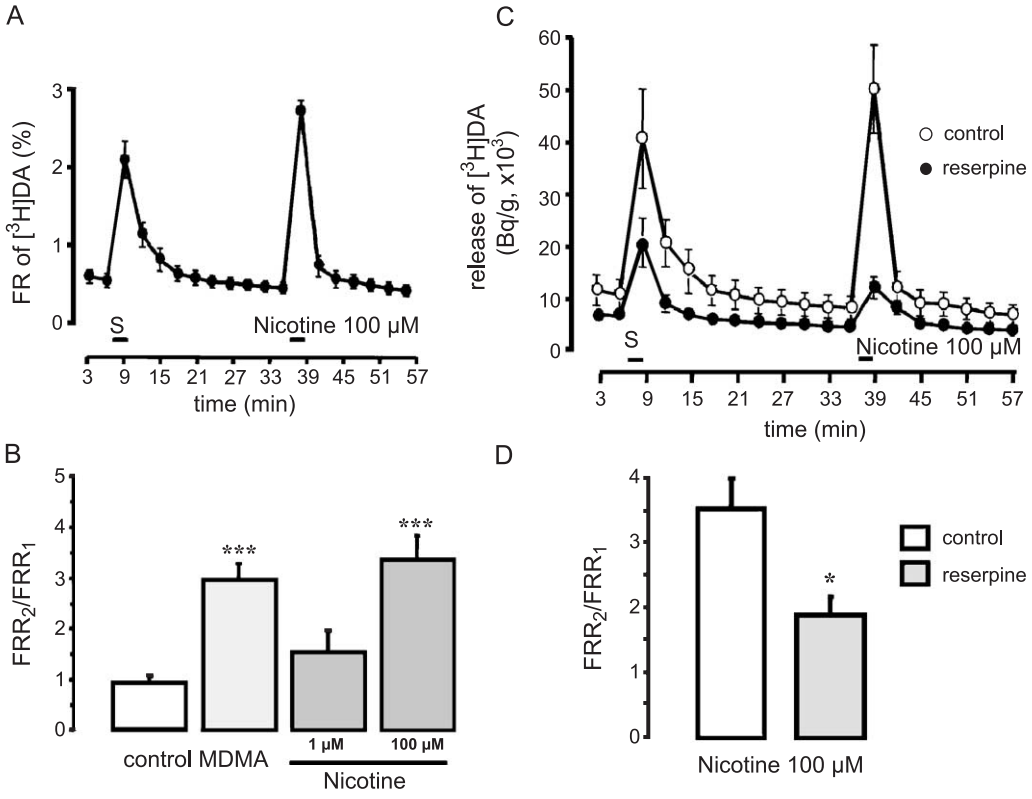

Fig. 5. Nicotine-evoked dopamine release of the nucleus accumbens in vitro. (A) Nicotine evoked a large dopamine release from the nucleus accumbens preparation. (B) Effects of MDMA and nicotine (in 1 and 100 μM concentrations) on the resting dopamine outflow. (C) Reserpine pretreatment 2 days prior to the experiments substantially decreased the dopamine-releasing action of nicotine. (D) Effects of nicotine on the resting dopamine outflow in control and reserpine-treated rats. Data presented are means±S.E.M.

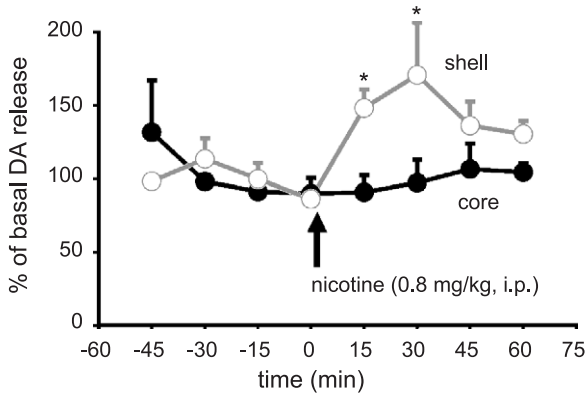

Fig. 6. Effect of nicotine on the in vivo dopamine release from the subregions of the nucleus accumbens. Core and shell regions were separately sampled. After the stabilization of baseline, the animals received nicotine (0.8 mg/kg, i.p. injection) at the time point 0, as indicated by the arrow, then four 15-min samples were collected. Data are the mean±S.E.M. of 4–6 independent experiments. One-way ANOVA with repeated measures followed by Dunnet's test was used for statistical comparison (*P<0.05).

tion of the slices obtained from reserpine-treated rats released much less radioactivity than from those of control rats, and the resting release was also decreased. The effect of 100 μM nicotine on the resting release of dopamine was significantly inhibited indicating that nicotine released dopamine from the vesicles through exocytosis (Fig. 5C,D).

3.5. Region-specific effect of nicotine in the nucleus accumbens in vivo microdialysis studies

The basal dopamine release was significantly higher in the core (13.83±1.24 pg/injection, P<0.05 two-tailed t-test) than in the shell region (7.71±0.48 pg/injection) of nucleus accumbens. Intraperitoneal injection of nicotine increased the dopamine release in the shell; the maximum effect (70% increase over the baseline) was reached 30 min after the injection, but the dopamine efflux remained elevated even after 60 min of a single injection (Fig. 6). In contrast, there was no change in the dopamine concentration in response to nicotine in the core region (Fig. 6). Similarly, Di Matteo et al. (2004) showed that nicotine enhanced the release of dopamine in the nucleus accumbens in conscious, freely moving rats.

4. Discussion

Perhaps the most important central effects of nicotine occur in the reward and cognitive systems. The GABA-containing interneurons of the hippocampus provide crucial inhibition of projection cells to control their excitability, which leads to the synchronization of network activity (Freund and Buzsaky, 1996). Acetylcholine release plays a major role in the rhythmical slow activity of the hippocampus underlying hippocampal learning and memory functions (Stewart and Fox, 1990). Main threads of current theories of nicotinic functions in the central nervous system (CNS) include that (i) nicotinic receptors may modulate, rather than mediate, fast synaptic transmission (McGehee and Role, 1995), most likely through extrasynaptic receptors, (ii) desensitisation of the nicotinic receptors, i.e. loss of function of the receptor, is an important mechanism underlying the central effect of nicotine during smoking and likely extends the computational power by nicotine, and (iii) the discovery, that nicotinic receptors directly release transmitters from presynaptic boutons skipping postsynaptic secondary modulations, highlights the importance of the release enhancement via a presynaptic nicotinic mechanism (Vizi and Lendvai, 1999). Because accumulation of evidence indicates that synaptic plasticity participates in memory and learning processes (Martin et al., 2000) and nicotinic receptors enhance plastic changes in several brain regions, we can conclude that the nicotinic enhancement of memory and learning function (Levin and Rezvani, 2000) is constructed on the level of cellular synaptic plasticity. In animal tests both acute and chronic nicotine treatment have been shown to improve working memory (Rezvani and Levin, 2001; Levin and Simon, 1998). Local infusions of mecamylamine into the hippocampus were found to impair working memory performance (Ohno et al., 1993). Both α4β2 and α7 nicotinic acetylcholine receptors are likely important for working memory function (Levin and Rezvani, 2000; Levin et al., 2002). Acetylcholine enhances and sharpens the cellular responses to the preferred stimulus while suppressing the less prominent responses to other modalities (Sarter and Bruno, 1997). It has been shown that the increased cholinergic activity (including both muscarinic and nicotinic mechanisms) is able to induce theta oscillation (4–12 Hz) so that synaptic plasticity becomes greatly sensitised (Huerta and Lisman, 1995; Cobb et al., 1999).

At the cellular level, activation of nicotinic acetylcholine receptors is able to induce large responses in various parts in the central nervous system, e.g. it upregulates ionotropic receptors (Risso et al., 2004). We have shown that hippocampal interneurons can be excited by nicotinic agonist resulting in large dendritic Ca^{2+} accumulations, which relatively homogenously distribute in the cellular compartments. This observation is well corresponding to the philosophy of nonsynaptic transmission: transmitters arriving from the extracellular matrix likely have little region specificity and exert tonic modulation on various cellular activities. Indeed, ultrastructural morphometric studies demonstrated that, in addition to monoaminergic varicosities, the normal cholinergic innervations of adult rat parietal cortex (Umbriaco et al., 1994) and hippocampus (Umbriaco et al., 1995) predominantly do not make synapses on other neurons (>85% of varicosities are nonsynaptic). These observations supported the idea that monoamines and acetylcholine participates mainly in nonsynaptic interaction (Descarries and Mechawar, 2000).

In our experiments backpropagating action potentials showed potentiation during the stimulation of nicotinic receptors by lowering the threshold of firing. When nicotinic stimulation was strong enough to produce continuous firing of the cells, high frequency trains were unable to evoke Ca^{2+} transients in the dendrites of hippocampal interneurons. These phenomena may occur in vivo when nicotine enters the brain and causes an initial excitement (perhaps through synchronizing network activity by coordinated activity of interneurons) but later responses show fading. Importance of backpropagating spikes and nicotinic modulation derives from the interplay between ongoing synaptic activity and traces of previous activities that may occur by the nonlinear summation of backpropagating action potentials and synaptic responses (Stuart and Sakmann, 1994; Stuart et al., 1997; Häusser et al., 2000). While hippocampal CA1 and neocortical pyramidal neurons show a distance-dependent reduction in the amplitude of subsequent backpropagating action potentials and in the evoked calcium transients in dendrites, stratum radiatum interneurons of the hippocampus exhibits incremental scaling (Stuart and Sakmann, 1994; Spruston et al., 1995, Golding et al., 2002; Rozsa et al., 2004).

Nicotinic currents can be more rapidly activated and desensitised in stratum radiatum interneurons (Sudweeks and Yakel, 2000). What function can be assigned to a receptor with fast desensitisation in extrasynaptic location, where a continuous, low tone of the agonist should be maintained? One might consider the following: acetylcholine released by a nearby cholinergic terminal would probably reach the extrasynaptic nicotinic acetylcholine receptors in lower and slowly changing concentrations. Because the recovery of nicotinic receptors is relatively fast, the asynchronous re-binding of acetylcholine would generate a long-lasting activity in the nicotinic receptor population causing slow fluctuation in Ca^{2+} level (Castro and Albuquerque, 1995). This mechanism may enable nicotinic acetylcholine receptors to influence plasticity. Desensitisation and activation of nicotinic acetylcholine receptors may co-exist during slow drug delivery: bath application of low-dose nicotine, which causes a great deal of receptor desensitisation in a few minutes, could also activate receptors and induce currents in limbic dopamine-containing cells (Pidoplichko et al., 1997; Pidoplichko et al., 2004). These data inspired the theory that nicotinic acetylcholine receptors located on interneurons may be the cellular correlates of the nicotine-induced cognitive enhancement (Jones et al., 1999).

Nicotinic acetylcholine receptors play an important role in the reward system. Our data provide evidence that the application of the abuse drug, MDMA, and nicotine results in a comparable dopamine-releasing effect in the nucleus accumbens but these effects are mediated via different mechanisms: while MDMA releases dopamine from the cytoplasm of nerve terminals, nicotine releases vesicular dopamine. The mesolimbic dopaminergic system originates mainly from the ventral tegmental area and projects to limbic areas such as the olfactory tubercle, the amygdala, the septum, and partly the nucleus accumbens. This part of the limbic system has been implicated in the reinforcing properties of drugs of abuse (Di Chiara and Imperato, 1988; Hemby et al., 1997; Corrigall et al., 1992; Pich et al., 1997; Giros et al., 1996; Pontieri et al., 1996; Di Matteo et al., 2004). On the other hand, the fact that presynaptic nicotinic acetylcholine receptors can directly release dopamine, i.e. “drive” the axon terminal, is worthy of consideration. Presynaptic action potential does not guarantee release (Huang and Stevens, 1997) so activation of presynaptic nicotinic acetylcholine receptors at the arrival of action potential into the axon terminal can increase the probability of release via the influence of nicotinic acetylcholine receptor-induced Ca^{2+} influx on the local signal integration. The activation of presynaptic nicotinic receptors could synchronize transmitter release (Sharma and Vijayaraghavan, 2003). Presynaptic nicotinic acetylcholine receptors play an important role in regulating the release of many neurotransmitters in different brain regions (Sandor et al., 1991; Lendvai et al., 1996, Kiss et al., 1999; Vizi and Lendvai, 1999; Kofalvi et al., 2000; Di Matteo et al., 2004; Vizi et al., 2004) with compound and region-specific mechanisms (Lendvai et al., 1996; Kiss et al., 1996; Kiss et al., 1997). Activation of nicotinic acetylcholine receptors in the hippocampus leads to release of noradrenaline in a $[Ca^{2+}]_o$-dependent and tetrodotoxin-sensitive way (Vizi et al., 1995; Vizi et al., 2004; Sacaan et al., 1995; Sershen et al., 1997). Neostigmine, a cholinesterase inhibitor, also enhances the extracellular level of noradrenaline measured by microdialysis, a method that samples from the extracellular space, therefore, provides data for the nonsynaptic release of transmitters (Kiss et al., 1999). Our data confirm previous findings on the effect of nicotine on dopamine release in the nucleus accumbens. We found that subcutaneous injection of nicotine induces an increase of dopamine concentration in the shell without affecting dopamine release in the core. The effect reaches the maximum within 30 min but an elevation of dopamine level can be observed even after 60 min. It has been shown that the dopamine response to nicotine is weaker in animals following chronic pretreatment with nicotine (Cadoni and Di Chiara, 2000). This suggests that in heavy smokers the rewarding effect of nicotine might last only for a few minutes because of the drop of dopamine concentration in the shell, which might provide a neurochemical basis for the phenomenon of chain-smoking. Since nicotine reaches the brain from the circulation, its concentration in the brain cannot be sufficient to activate low-sensitivity nicotinic acetylcholine receptors located within the synapse, but might activate high-sensitivity extrasynaptic receptors. Therefore, it is suggested that these receptors are responsible for the central action of nicotine and might be primary targets for drugs in smoking cessation.

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