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NICOTINIC AND MUSCARINIC MODULATIONS OF EXCITATORY SYNAPTIC TRANSMISSION IN THE RAT PREFRONTAL CORTEX *IN VITRO*

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Abstract—The importance of the cholinergic innervation of the neocortex in cognitive functions has been shown in a number of clinical and animal studies. Until recently, attempts to study the mode of action of acetylcholine in the neocortex have concentrated on muscarinic effects, whereas cholinergic actions mediated by nicotinic receptors have been difficult to demonstrate. The present work was undertaken to study the mechanism of action of nicotinic agents on cortical neurons and compare it to muscarinic effects by means of intracellular recordings in a slice preparation. The study was performed in the prelimbic area of the rat prefrontal cortex, a cortical region particularly involved in cognitive processes. Recordings were made from pyramidal cells located in layers II/III and synaptic potentials were evoked by stimulation of superficial cortical layers. Iontophoretic applications of nicotinic agonists (nicotine, dimethylphenylpiperazinium, cytisine) increased the amplitude of the monosynaptic excitatory postsynaptic potential mediated by non-*N*-methyl-D-aspartate glutamate receptors in 14% (22/159) of cells. This effect was abolished by the selective nicotinic blocker, neuronal bungarotoxin ($IC_{50} = 0.6$–$0.7\ \mu M$) and by dihydro-β-erythroidine ($IC_{50} = 20$–$30\ \mu M$), whereas hexamethonium, mecamylamine, curare and α-bungarotoxin were ineffective. The nicotinic agonists did not change resting membrane potential, input resistance or current–voltage relationship. They also did not affect the depolarizations produced by glutamate applied by iontophoresis in the somatic or dendritic area. In contrast, the muscarinic agonists (muscarine, acetyl-β-methylcholine) decreased the amplitude of the excitatory postsynaptic potential in 100% of the neurons tested. Atropine was more effective ($IC_{50} = 0.08\ \mu M$) than pirenzepine ($IC_{50} = 2\ \mu M$) to antagonize the muscarinic action. These effects were observed in the absence of any direct postsynaptic change in membrane potential or input resistance, provided that the site of the iontophoretic application was more than 100 μM distant from the soma. The muscarinic agonists did not influence the actions of iontophoretically applied glutamate.

These results suggest that nicotinic and muscarinic agonists modulate excitatory synaptic transmission mediated at dendritic sites by non-*N*-methyl-D-aspartate glutamate receptors, possibly through a presynaptic action. Thus ascending cholinergic systems may take part in information processing in the prefrontal cortex through the control of ongoing excitation to pyramidal cells.

Over the past decade, a substantial body of research in animals and humans has revealed the role of the telencephalic cholinergic system in higher brain functions (for reviews, see Refs 3,7,10). This concept has been reinforced by the discovery that senile dementia of Alzheimer type is associated with a loss of the cortical cholinergic innervation originating from the basal forebrain. In animal models of the disease, damage to cholinergic nuclei of the basal forebrain produces deficits in some forms of learning and memory, particularly in those requiring sustained attention.[31,36,37] Similar behavioral impairments have been observed after lesion of the prefrontal cortex in primates and rats.[4,12,34] Thus, the contribution of the frontal cortical area to the cognitive deficits after lesion of forebrain cholinergic systems might be of particular importance.

Attempts to ameliorate performance following basal forebrain lesions by cholinergic drugs or cholinergic transplants suggest the involvement of both muscarinic and nicotinic actions of acetylcholine (ACh) in cognitive functions.[16,29,31] In Alzheimer brains, research has focused predominantly on the status of muscarinic binding in the neocortex, however, specific changes have been difficult to demonstrate.[30] In contrast, a consistent loss of nicotinic binding sites has been observed, particularly in frontal cortical areas (see references in Ref. 22). Whether these sites represent functional nicotinic receptors has long been doubtful because of the lack of specific ligands for neuronal nicotinic acetylcholine receptors (AChRs). In a recent study in the rat prefrontal cortex,[48] we have been able to demonstrate with the use of neuronal bungarotoxin, a selective blocker for some neuronal nicotinic AChRs,[24] that ACh and nicotinic agonists enhance extracellular field potentials by acting specifically on nicotinic AChRs. The present work was undertaken

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Abbreviations: ACh, acetylcholine; AChR, acetylcholine receptor; APV, D,L-2-amino-5-phosphonovaleric acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DHBE, dihydro-β-erythroidine; DMPP, dimethylphenylpiperazinium; EPSP, excitatory postsynaptic potential; eEPSP, early EPSP; IPSP, inhibitory postsynaptic potential; NBT, neuronal bungarotoxin; NMDA, *N*-methyl-D-aspartate; PSP, postsynaptic potential.

to study the mechanism of action of nicotinic agonists on prefrontal pyramidal cells and compare it to the effects of muscarinic agents by means of intracellular recordings.

EXPERIMENTAL PROCEDURES

Male Sprague–Dawley rats from Iffa Credo, l'Arbresles, France (100–200 g) were decapitated under ether anesthesia and the brains were rapidly removed. Transverse slices (500 μm thick) were prepared from the medial prefrontal cortex[49] and placed in a recording interface chamber (Haas type, Medical System). Slices were perfused with artificial cerebral spinal fluid at a constant flow of 1.5 ml/min. The perfusion medium contained in mM: NaCl, 124; KCl, 3.5; NaH_2PO_4, 1.25; $MgSO_4$, 1.3; $CaCl_2$, 2.5; $NaHCO_3$, 26; glucose, 10, gassed with 95% O_2/5% CO_2 at 36.5°C.

Stable intracellular recordings of duration 1–4 h were made from layers II/III neurons with glass microelectrodes filled with 4 M potassium acetate (resistance 70–120 MΩ). All potential recordings and current injections were performed using the switched current clamp mode of the amplifier (Axoclamp 2A) and displayed directly on a pen recorder (Gould RS 3400). Unless stated, during recordings of long duration, the maximum variation of resting potential allowed was 2 mV. A bipolar tungsten stimulating electrode (tip separation 100 μm) was placed in the superficial cortical layers at 250–300 μm away from the recording site and orthodromic responses were elicited by brief stimuli (0.02 ms) of various intensities (1–50 μA). Cholinergic agonists and other drugs were applied by iontophoresis (Neurophore-BH-2, Medical System Corp.) from a five-barrelled pipette (barrel resistances 20–100 MΩ). Retaining currents of 7–20 nA were used to prevent leakage of the drugs and current flow was automatically balanced. The solutions used for iontophoresis were as follows: acetylcholine Cl (1 M, pH 4), acetyl-β-methylcholine Cl (0.5 M, pH 4.5), dimethylphenylpiperazinium iodide (DMPP, 1 M, pH 5), cytisine (1 M, pH 4.5), L-glutamate (0.2 M, pH 8), muscarine Cl (0.05 M, pH 5), NaCl (1 M), nicotine hydrogen tartrate (0.05 M, pH 4), all from Sigma, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 1 mM, pH 8, RBI). The effectiveness of the agonists was systematically assessed on the extracellular field potential evoked in layers II–III, as already described.[48] Control experiments for current or pH artifacts were routinely carried out using NaCl solutions adjusted to the pH of the drug solutions. Other drugs were applied in the bathing medium: atropine SO_4, (0.01–10 μM), D,L-2-amino-5-phosphonovaleric acid (APV, 30 μM), α-bungarotoxin (0.3–3 μM), hexamethonium Cl (0.1–1 mM), mecamylamine HCl (0.1–1 mM), pirenzepine (0.1–10 μM), scopolamine aminoxyde (0.01–10 μM), *d*-tubocurarine Cl (50 μM), all from Sigma, CNQX (10 μM, RBI), neuronal bungarotoxin (NBT, 0.1–1.6 μM, Biotoxins Inc.). Dihydro-β-erythroidine (DHBE, 1–100 μM) was donated by Merck, Sharp and Dohme. The data were recorded on videotape and analysed with the program pClamp, version 5.5, by Axon Instruments (Burlingame, CA). All measurements are given as mean ± S.D.

RESULTS

Effects of nicotinic and muscarinic agonists on postsynaptic potentials

Stable intracellular recordings were obtained from neurons (n = 159) located exclusively in layers II/III of the prelimbic region of the medial prefrontal cortex[49] (Brodmann's area 32). All neurons possessed physiological properties typical for neocortical pyramidal cells: their membrane potential was −78.1 ± 5.0 mV (n = 132), action potential amplitude measured from spike threshold 77.6 ± 6.7 mV, input resistance 24.5 ± 5.6 MΩ (measured at the plateau of the voltage response to intracellular injection of negative current pulses, 0.2–0.4 nA, 150 ms, from membrane potential of −80 mV). As already described in rodent frontal cortex (see references in Ref. 43),

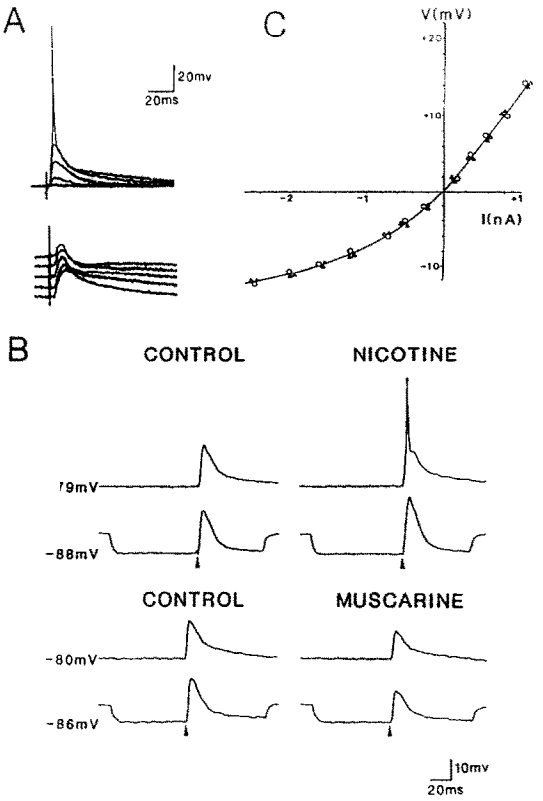

Fig. 1. Opposite effects of nicotine and muscarine on subthreshold PSPs in layers II/III pyramidal neurons of the prefrontal cortex. (A) Some characteristics of synaptic potentials evoked by stimulation of superficial cortical layers. The top traces show the development of PSPs with increasing stimulus strength. The threshold stimulation produced on overshooting spike, resting membrane potential (RP) = −79 mV. Lower traces show a subthreshold PSP recorded at different membrane potentials in another cell, resting membrane potential = −78 mV. Depolarization to levels less negative than −70 mV uncovered a hyperpolarizing component reflecting the composite EPSP–IPSP sequence of the PSP. (B) The control recordings show subthreshold PSPs evoked at resting membrane potential and during a hyperpolarizing pulse. During the iontophoretic application of nicotine (50 nA, 15 s), the same stimulus elicited a spike from resting membrane potential and increased the PSP amplitude at the hyperpolarized potential. In contrast, muscarine (30 nA, 15 s) decreased the PSP amplitude without any significant effect on resting membrane potential and input resistance. In both cases, the iontophoretic pipette was located in the region of the apical dendrite, at about 150 μm from the soma. Each trace is an average of three to six consecutive records. The stimulus artefact is indicated by an arrow. (C) I/V curve generated by intracellular injection of current pulses (150 ms, measurements taken at the plateau of the voltage response). Neither nicotine nor muscarine produced any significant effect on the current–voltage relationship of the cell. ○, control; ▲, nicotine; △, muscarine.

pyramidal neurons exhibited regular spiking activity upon depolarization and showed a non linear current-voltage relationship reflecting inward rectification in depolarizing and hyperpolarizing directions.

The stimulation of the superficial layers evoked a depolarizing postsynaptic potential (PSP; Fig. 1A) graded in amplitude with increasing stimulus intensity and exhibiting the major characteristics of PSPs recorded in layers II/III of cortical slices.[2,8,14,18,42,44] Typically, stimulation with low intensities first elicited excitatory postsynaptic potentials (EPSPs). When higher stimulus strengths were used, EPSPs were followed at short latencies by a presumed GABA-mediated inhibitory postsynaptic potential (IPSP). Because of the high negative values of resting membrane potentials of neocortical neurons *in vitro*, the early IPSP appeared as a depolarizing potential which reversed to a hyperpolarization upon membrane depolarization. The average reversal potential of the IPSP was −70 mV, a value corresponding to the equilibrium potential for Cl ions.[2,8,18]

The iontophoretic application of nicotine either near the recording site or in the region of the apical dendrite, increased the PSP amplitude and triggered spikes in 14% of cells (22/159). Similar effects were observed in these cells with the nicotinic agonists, DMPP ($n = 5$) and cytisine ($n = 4$). A typical example of the action of nicotine is illustrated in Fig. 1B: in the control recording, a composite PSP was evoked by a stimulus just below spike threshold. During nicotine application (50 nA for 15 s), the same stimulation evoked a spike at resting membrane potential and a marked increase (+23%) in the amplitude of the PSP at the hyperpolarized potential. None of the nicotinic agonists produced any observable change in resting membrane potential, input resistance, spike threshold and current-voltage relationship (Fig. 1C).

In contrast, the muscarinic agonists muscarine ($n = 71$), and acetyl-β-methylcholine ($n = 47$) reduced the peak amplitude of the subthreshold PSP. This effect was observed in the absence of consistent changes in membrane potential and input resistance, provided that the iontophoretic application was located in the apical dendritic region more than 100 μm distant from the soma (see below). With closer applications, the muscarinic agonists typically produced a membrane depolarization and increase in input resistance, as described in the neocortex.[20,27,28] All cells which were affected by nicotine were also sensitive to the muscarinic agonists.

As compared with the effects of nicotinic and muscarinic agonists, ACh produced inconsistent changes in PSP amplitude ($n = 10$, not illustrated). A similar observation has been mentioned by McCormick and Prince.[28] The present results showing an opposition between nicotinic and muscarinic actions may account for the inconsistent effects of ACh on PSPs.

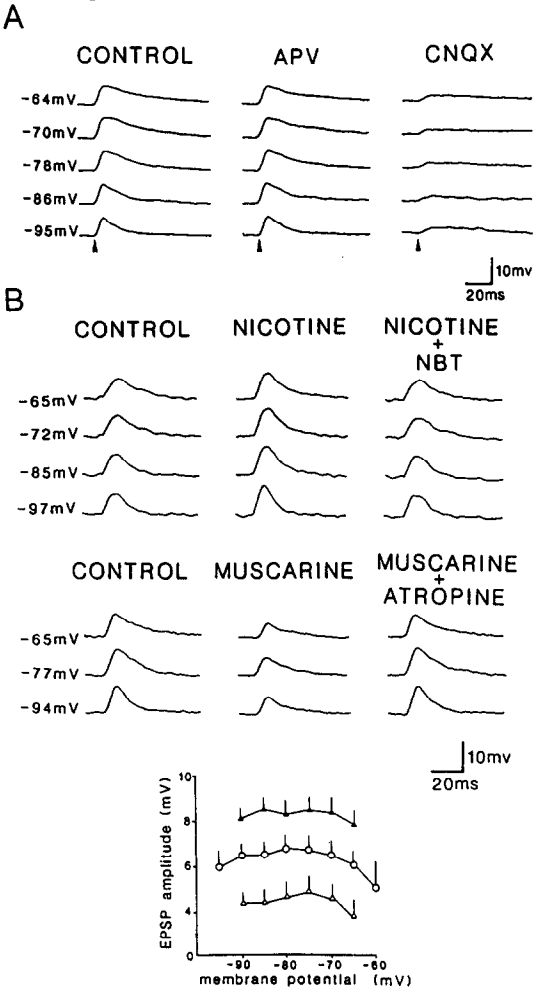

Fig. 2. Effects of nicotine and muscarine on the early EPSP (eEPSP). (A) Characteristics of the early EPSP showing the involvement of non-NMDA glutamate receptors. The early EPSP was evoked at different membrane potentials shifted by depolarizing and hyperpolarizing current pulses (500 ms, resting membrane potential = −78 mV). Note the apparent voltage independence of the early EPSP indicating its distal dendritic origin. Variations in decay reflect the non-linearity of the somatic I/V relationship and the cable properties of the dendrites. Bath application of APV (30 μM, 20 min) had no measurable influence on the early EPSP, whatever the membrane potential. The addition of CNQX (10 μM) produced a powerful block of the early EPSP after 20 min perfusion. Note that the remaining component was not affected by shifting the membrane potential. Each trace is an average of eight to 16 successive events. (B) Nicotine (50 nA, 15 s) increased the amplitude of the early EPSP whatever the membrane potential value (resting membrane potential = −80 mV). Bath application of NBT (1.6 μM, 30 min) produced a complete blockade of the effect of nicotine. In another cell, the iontophoretic application of muscarine (25 nA, 15 s) in the apical dendritic area reduced the early EPSP amplitude. The effect was antagonized by perfusion with atropine (10 μM, 15 min) and full recovery occurred after 20–30 min washout. Each trace is an average of three to six successive records (resting membrane potential = −77 mV). The graphical representation of the effects of nicotine and muscarine measured the peak amplitude of the early EPSP at various levels of membrane potential. Each point represents the mean values (±S.D.) of the early EPSP amplitudes recorded in four cells, each individual neuron being tested alternatively for the effects of nicotine and muscarine. ○, control; ▲, nicotine; △, muscarine.

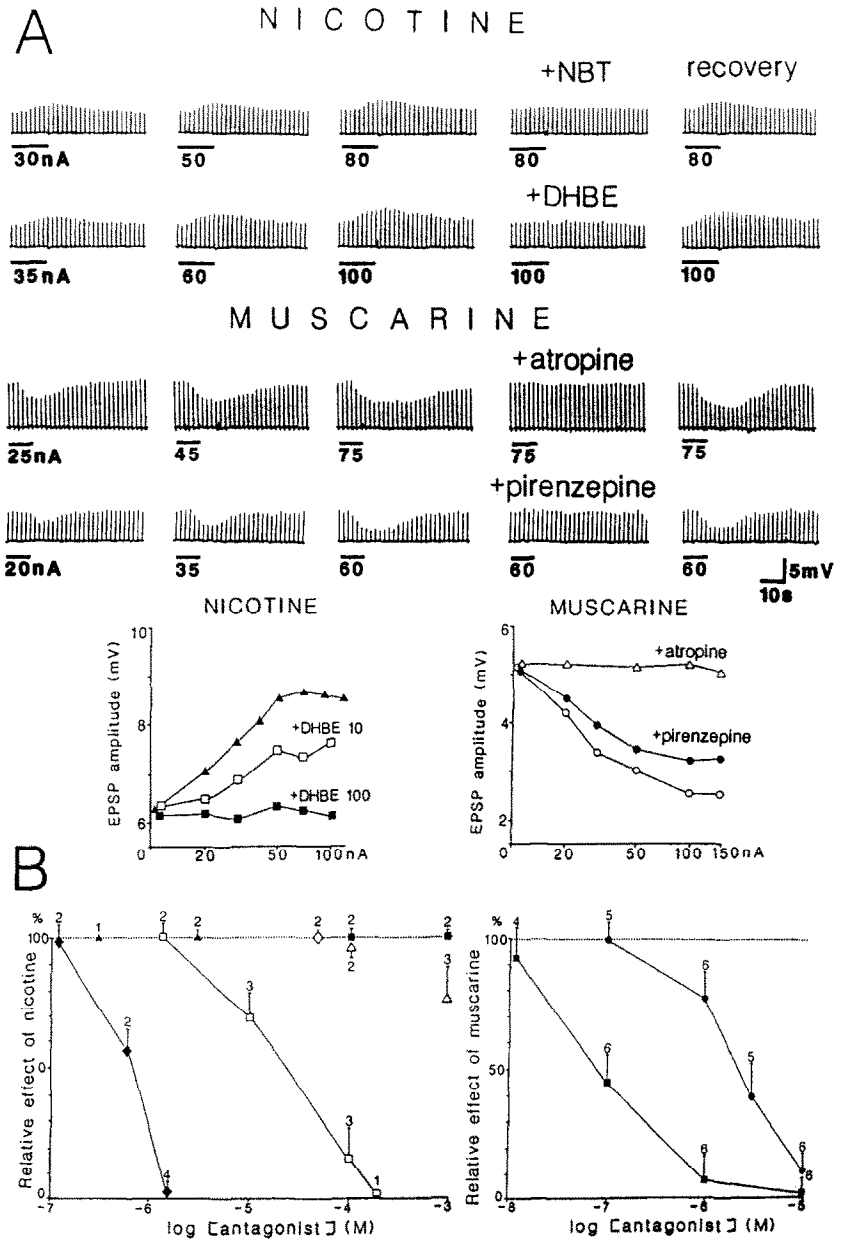

Fig. 3. Pharmacological characteristics of the effects of nicotine and muscarine on the eEPSP. (A) Time-courses and dose-dependent effects of nicotine and muscarine applied by iontophoresis in the apical dendritic area. Upper part: continuous chart recordings depicting the effects of various doses of the cholinergic agonists on the eEPSP (upward deflection). The increase in eEPSP amplitude produced by nicotine was blocked by bath application of NBT (1.6 μM, 30 min) and recovered after 100 min washout (resting membrane potential = −77 mV). In another neuron, DHBE (100 μM) antagonized the effect of nicotine after 15 min perfusion. Full recovery occurred after 30 min washout (resting membrane potential = −81 mV). Muscarine produced a dose-dependent decrease in the eEPSP amplitude. The effect was totally blocked by atropine (1 μM, 12 min) and recovered after 12 min washout (resting membrane potential = −75 mV). A concentration of 10 μM pirenzepine for 15 min was necessary to produce a full block of the muscarine effect. Recovery was observed after 20 min washout (resting membrane potential = −80 mV). Lower part: graphical representation of the effects of nicotinic and muscarinic agents on individual cells. Left: measurements of the eEPSP amplitude showing the effect of increasing doses of nicotine (▲) and the antagonism by bath perfusion of two concentrations of DHBE, 10 μM (□) and 100 μM (■) in the same cell. Right: graph showing the reduction of the eEPSP amplitude by muscarine (○) and the differential block by 1 μM pirenzepine (●) and 1 μM atropine (△). (B) Dose–response curves for nicotinic and muscarinic agonists and antagonists. Left: effects of various nicotinic antagonists on the relative increase of eEPSP by nicotine. NBT (◆) and DHBE (□) blocked the nicotinic effect but not α-bungarotoxin (▲), hexamethonium (■), curare (◇) and mecamylamine (△). The slight effect of 1 mM mecamylamine is considered as nonspecific. Right: dose-response curves for the antagonism by pirenzepine (●) and atropine (■) of the muscarinic depression of the eEPSP. The number of cells averaged are indicated for each point.

Effects of nicotinic and muscarinic agonists on the early excitatory postsynaptic potential

We next attempted to analyse the mechanism of action of the cholinergic agents on an identified component of the PSP, i.e. the early EPSP.

Characterization of the early excitatory postsynaptic potential. EPSPs could be evoked in isolation in all pyramidal cells by using stimulus intensities subthreshold for the activation of IPSP, i.e. membrane depolarization did not uncover any hyperpolarizing potential (Fig. 2A). The lowest intensity stimuli evoked an early EPSP (eEPSP) with short onset latencies (2–3 ms), time to peak of 4.6 ± 1.0 ms and peak amplitude of 6.8 ± 2.2 mV ($n = 108$). The eEPSP followed high stimulation rates with stable latency and so was considered as monosynaptic.

In all neocortical slice preparations studied so far, EPSPs evoked in layers II/III pyramidal cells by stimulation of intracortical layers or underlying white matter, were found to be mediated by excitatory aminoacids (glutamate, aspartate) acting on *N*-methyl-D-aspartate (NMDA) and/or non-NMDA receptors.[2,14,42,44] Since cholinergic agonists might differentially affect NMDA and non-NMDA responses, it was important to further characterize the eEPSP by investigating its voltage dependency and sensitivity to APV and CNQX, the respective blockers of NMDA and non-NMDA receptors.[17,51] In the example illustrated in Fig. 2A, it is shown that shifting the membrane potential from -95 mV to -64 mV was not accompanied by any marked change in the amplitude of the eEPSP. Bath application of 30 μM APV for 20 min, a concentration which blocked the response to iontophoretically applied NMDA (not shown), did not produce any measurable effect on the eEPSP whatever the membrane potential. In contrast, CNQX (10 μM for 20 min) was a potent blocker of the eEPSP. These results strongly suggest that the eEPSP is mediated by non-NMDA receptors, most probably located at dendritic sites remote from the soma (see references in Refs. 14,42).

Effects of nicotinic agonists on the early excitatory postsynaptic potential. In neurons where nicotine was found to increase subthreshold PSPs ($n = 22$), nicotine also produced a comparable increase in the amplitude of the eEPSP (Fig. 2B). This effect was accompanied by a shorter time to peak and was not obviously influenced by changes in the membrane potential. Bath-applied APV (30 μM, 20 min, $n = 3$) did not influence the effect of nicotine on the eEPSP. Thus, there was no evidence for any possible effect of nicotine on an undetected NMDA contaminant of the eEPSP.

The effect of nicotine was fully reversible and dose-dependent. Its time course was relatively slow in onset (5–12 s), reached a maximum within 15–20 s and persisted for about 10–20 s after the application (Fig. 3A). No fading of the response was observed when periods of 30–60 s elapsed between successive applications. The effects of DMPP ($n = 5$) and cytisine ($n = 4$) were not markedly different from those of nicotine ejected from the same pipette (not shown). No special attempt was made to study the relative potencies of the agonists as different iontophoretic barrels have different releasing properties, thus making the comparison of the results difficult.

The pharmacology of the nicotinic effect on the eEPSP was further investigated by adding antagonists to the perfusion medium (Figs 2B,3). The nicotinic blocker, NBT completely antagonized the effects of nicotine and DMPP at a concentration of 1.6 μM ($n = 4$), half inhibition (IC_{50}) being about 0.6–0.7 μM ($n = 2$). The action of NBT was not easily reversible as only partial recovery needed more than 90 min washout (Fig. 3A). The effect of the nicotinic agonists on the eEPSP were also inhibited by DHBE ($IC_{50} = 20$–30 μM, $n = 3$), an antagonist known to block nicotinic responses in both muscle and autonomic ganglia. Full reversal of DHBE action was observed after 20–30 min washout. In contrast, the other classical antagonists tested, i.e. hexamethonium (100 μM, $n = 2$; 1 mM, $n = 2$), curare (50 μM, $n = 2$) and mecamylamine (100 μM, $n = 2$; 1 mM, $n = 3$) were inactive. The selective blocker of skeletal muscle nicotinic AChRs, α-bungarotoxin (0.3–3 μM, $n = 2$) was without effect when applied for up to 30 min.

Effects of muscarinic agonists on the early excitatory postsynaptic potential. In experimental conditions where the muscarinic agonists, muscarine ($n = 71$) and acetyl-β-methylcholine ($n = 47$), reduced the subthreshold PSP (see above), we observed a decrease in amplitude of the eEPSP (Fig. 2B). Varying the membrane potential did not markedly influence the reduction of the eEPSP. The effect was maximal 10–15 s after the beginning of application and persisted for 10–20 s after termination (Fig. 3A). The addition of the muscarinic antagonists, atropine (Figs 2B,3) and scopolamine (not shown) blocked the effect of the agonists with an IC_{50} of about 0.08 μM ($n = 6$). Pirenzepine, another muscarinic antagonist, was used to distinguish between M_1 and M_2 muscarinic AChRs subtypes to which it is known to bind with high and low affinity, respectively.[15] With an IC_{50} value around 2 μM (Fig. 3B, $n = 6$), pirenzepine was 30–40 fold less effective than atropine, suggesting the involvement of M_2 muscarinic AChR subtype.

Site of action of cholinergic agonists. The observation that the modulations of the eEPSP by the cholinergic agonists were not necessarily associated with measurable changes of membrane potential or input resistance raised two major questions: (i) were the cholinergic agonists acting too far away from the soma to be seen by the recording electrode and thus, was it possible to find an optimal location for the iontophoretic electrode? (ii) were the responses to iontophoretic pulses of glutamate affected by the cholinergic agonists in the same way as the eEPSP? These questions were addressed by comparing the effects of nicotine and muscarine on the eEPSP and on

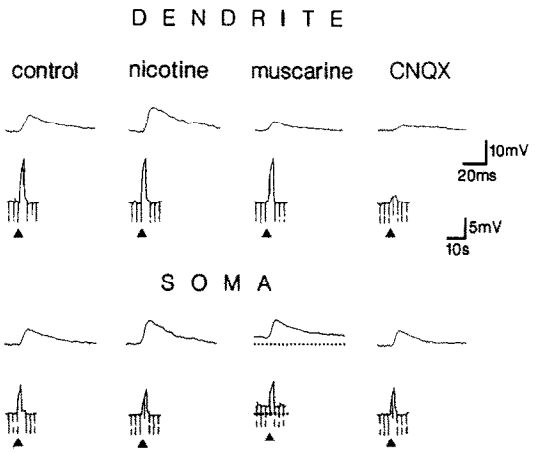

Fig. 4. Comparison of the effects of nicotine and muscarine on the eEPSP and on depolarizations produced by glutamate. With the iontophoretic pipette located in the apical dendritic area, nicotine (100 nA, 12 s) and muscarine (35 nA, 12 s), respectively, increased and decreased the eEPSP but did not affect the depolarizations produced by coapplication of glutamate (50 nA, 1 s). CNQX applied from the same pipette (3 nA, 12 s) strongly depressed both the eEPSP and glutamate depolarization. When the iontophoretic pipette was moved to the somatic area, the same currents of nicotine as before (100 nA, 12 s) produced a similar increase in the eEPSP. Low currents of muscarine (10 nA, 6 s) produced a slight depolarization from RP (−78 mV) without obviously affecting the eEPSP (the same currents applied in the dendritic area reduced the eEPSP). The depolarizations produced by glutamate (50 nA, 1 s) were smaller than before (see text). They were not changed by coapplied nicotine or muscarine. Previously effective currents of CNQX (3 nA, 12 s) did not alter the eEPSP nor glutamate depolarizations. All recordings are from the same cell.

the depolarizations produced by glutamate applied at a distance or close to the soma. The experiments were performed thoroughly in four cells and partly in one cell, all exhibiting similar behavior as illustrated in Fig. 4.

With the iontophoretic pipette located in the presumed area of the apical dendrite (more than 100 μm from the recording site), nicotine (50–120 nA, 10–15 s) and muscarine (25–75 nA, 10–15 s) respectively increased and reduced the eEPSP amplitude without any visible effect on membrane potential or input resistance as described above. Short pulses of glutamate (1–2 s, 20–60 nA) ejected from the same pipette produced brief membrane depolarization (6–12 mV). The concomittant application of effective currents of nicotine or muscarine did not cause any obvious change of glutamate responses. Small amounts of CNQX (2–6 nA, 10–15 s) applied with the same iontophoretic electrode strongly depressed both the eEPSP and glutamate depolarization (−70 to −90%, $n = 5$). These results suggest that (i) the active synapses generating the eEPSP were located proximal to the apical dendrite and (ii) the cholinergic agonists did not alter the eEPSP via an interaction with dendritic non-NMDA receptors.

After recovery from CNQX effect (20–30 min), the iontophoretic electrode was positioned as close to the soma as possible (within 20–30 μM), taking care not to alter resting membrane potential. The ejection of nicotine increased the eEPSP in the same way as observed with the dendritic application ($n = 5$). Similar currents of nicotine (100–120 nA, 12–15 s) were necessary to evoke the maximum effect. The application of small amounts of muscarine (10–25 nA, 5–8 s) close to the soma produced limited and steady membrane depolarization (2–5 mV) and no marked change in the eEPSP amplitude, contrasting with the dendritic site where similar currents had depressed already the eEPSP. Increasing the time and amount of muscarine ejection produced repetitive firing (not shown), an effect never observed during the dendritic application.

Moving the site of glutamate ejection close to the soma produced smaller depolarizations (2–6 mV) as compared with distal applications using similar currents. In three out of five cells, the depolarization was associated with a small decrease in input resistance. Nicotine applied concomittantly did not interfere with glutamate responses, neither did small amounts of muscarine producing limited membrane depolarization as described above. The iontophoretic application of previously effective currents of CNQX did not alter the eEPSP ($n = 4$). The probable distal origin of the eEPSP may account for this observation. In addition, small amounts of CNQX were ineffective to produce any significant reduction of the size of glutamate depolarization ($n = 4$). Similar results have been reported by Thomson[45] and support the view that, in control conditions, glutamate responses of pyramidal cells mainly involve non-NMDA receptors located at distal sites from the soma.

DISCUSSION

Nicotinic and muscarinic modulations of synaptic potentials in the prelimbic cortex

We chose to study the cholinergic sensitivity of pyramidal cells in layers II/III of the prelimbic region of the medial prefrontal cortex for two main reasons: (i) the prelimbic area in the rat is considered to be the closest to the prefrontal cortex of primates, anatomically and functionally;[47] (ii) in all cortical areas so far examined, nicotinic binding sites are concentrated in upper and middle layers.[5,35,39] The equivalent layers in the prelimbic cortex, which is an agranular cortex lacking layer IV, are layers II/III. Muscarinic binding sites are also abundant in these layers as well as cholinergic terminals.[26,30,39]

The synaptic potentials recorded in the prelimbic region were similar in many respects to those described in layers II/III of various cortical areas, although differences were reported depending on the stimulation site, i.e. the superficial cortical layers or the underlying white matter. In our preparation, the characteristics of the EPSP evoked at short latency (eEPSP) by low-intensity stimulation of upper layers closely resembled those reported by Hablitz and

co-workers[14,42] in the anterior cingulate cortex, a frontal area proximal to the prelimbic cortex. In both studies, there is strong evidence that the eEPSP is monosynaptic and mediated by glutamate non-NMDA receptors located in the apical dendritic region.

We observed that the iontophoretic application of cholinergic agonists altered the eEPSP amplitude in two opposite directions: nicotinic agonists potentiated the eEPSP, while muscarinic agonists reduced it. That these actions were not necessarily associated with a measurable effect on membrane potential or input resistance raised the question of the possible site of the cholinergic actions. The comparison between the effects of nicotine and muscarine on the eEPSP and on depolarizations produced by glutamate suggests sites of action remote from the soma. The nicotinic agonists were found to increase the eEPSP without producing any visible postsynaptic effect whether applied to the somatic or to the dendritic area. This, together with the relatively slow time-course of the nicotinic effects (10–20 s), suggest that the nicotinic receptors influencing the eEPSP generation may be distributed in remote sites not easily accessible. The mapping of the sensitivities to glutamate and CNQX along the neuron showed the predominance of non-NMDA receptors in the dendritic tree, in agreement with previous studies.[14,42,45] As compared with the nicotinic effects on the eEPSP, the depolarizations produced by glutamate applied in the somatic or dendritic area were not altered by the coapplication of nicotine. The most parsimonious interpretation of the present results is that nicotinic agonists increase the efficiency of excitatory transmission mediated at dendritic sites by non-NMDA glutamate receptors, possibly through a presynaptic action. The same interpretation would hold for the effects of muscarinic agonists on the eEPSP, the reduction of which appears as the reverse image of the nicotinic facilitation. In addition, the presumed presynaptic effect of muscarine was clearly dissociated from the direct postsynaptic effects located on the soma.[20,28] Similar characteristics of the muscarinic actions have been reported in the hippocampus (see references in Ref. 52) and thus appear as a common feature of both neo- and archeocortices. It would not be the case for nicotinic actions which have not been detected in the hippocampus by intracellular recordings.[6]

Although several arguments point towards presynaptic cholinergic actions, we have no direct proof and so we cannot exclude a remote postsynaptic effect not detectable by the recording electrode located in the soma. Small changes in conductance at distal sites may alter the eEPSP amplitude. A decrease in resistance, as expected from the activation of nicotinic receptors, would result in a smaller eEPSP while a larger eEPSP would reflect an increase in resistance triggered by muscarinic receptors. Obviously, our results do not favour this hypothesis.

The low percentage (14%) of cells sensitive to nicotine is difficult to explain. It is possible that our observations concern only one among the many putative subtypes of nicotinic AChRs which seem to co-exist in the CNS.[40] Another important question concerns the morphology of cholinergic terminals and synapses in the neocortex. According to recent ultrastructural analysis,[46] cholinergic axonal varicosities would be mostly non junctional, thus making crucial the questions of the repartition and accessibility of the cholinergic receptors.

The cellular localization of nicotinic AChRs in the neocortex is still unknown. In frontal cortical areas, nicotinic binding sites are concentrated in upper layers.[5,35,39] Recent autoradiographic data (P. Clarke, unpublished observations) have shown that lesions of the mediodorsal thalamus, the source of massive projections to layers II/III of the prefrontal cortex, produced a significant decrease in binding signals for nicotine. The presence of nicotinic AChRs on thalamo-cortical afferents,[35,39] which are likely to contain excitatory amino acids, may well account for our results. There is also good evidence for the localization of muscarinic receptors on thalamo-cortical terminals in the anterior cingulate cortex.[1,50]

Possible mechanisms of putative presynaptic cholinergic actions

Our observations are in line with data pointing to the existence of presynaptic nicotinic AChRs in many brain areas. Biochemical studies using brain slices or synaptosomes have shown that nicotinic AChRs facilitate the liberation of various neurotransmitters in a Ca^{2+}-dependent manner (for review, see Ref. 54). It has been proposed that Ca^{2+} influx through presynaptic nicotinic AChRs could directly trigger the liberation of the neurotransmitter.[19,32,54] At variance with the biochemical approach, our experiments are concerned with neurotransmitter release in response to spike invasion in afferent terminals. The possibility of a depolarization of presynaptic endings by nicotine would not favour action potential invasion and subsequent synaptic release. Recently, Wong and Gallagher[53] have described in the dorsolateral septum a "novel class" of nicotinic AChR mediating long lasting hyperpolarizations. Such a receptor type located presynaptically may well account for an increased neurotransmitter release by nicotine. Interestingly, the pharmacological properties of the nicotinic effects in cortical and septal neurons are very close. An alternative mechanism has been proposed recently by Lena *et al.*[21] in a patch-clamp study in the interpeduncular nucleus. Their data suggest that presynaptic nicotinic AChRs located on the preterminal portions of axons (and not on synaptic boutons) would control transmitter release by directly triggering spike discharge in these axons.

Several mechanisms have also been suggested to account for the reduction of transmitter release by muscarinic agonists.[33,52] The activation of the presynaptic muscarinic AChRs would produce a

depolarization of afferent terminals, thus making action potential invasion less efficient in the liberation of the neurotransmitter. Another possibility may be a decrease in the Ca^{2+} conductance involved in stimulus–secretion coupling.

Pharmacology of cholinergic effects on the early evoked excitatory postsynaptic potential

In the rat CNS, the genes encoding nicotinic AChRs correspond to five putative agonist binding subunits ($\alpha 2$–$\alpha 6$) and to four non-binding subunits ($\beta 2$–$\beta 5$) (for reviews, see Refs 13,40). Expression studies in oocytes[25] have revealed that the nicotinic AChRs formed with the combinations $\alpha 3\beta 2$ and $\alpha 4\beta 2$ are blocked by NBT, whereas $\alpha 2\beta 2$ and $\alpha 3\beta 4$ are not. Thus the nicotinic AChRs mediating the effects described above may be constituted by the combinations $\alpha 3\beta 2$ and/or $\alpha 4\beta 2$.

Our pharmacological analysis has shown that the nicotinic effects on the eEPSP were blocked by NBT and DHBE, whereas classical nicotinic blockers like hexamethonium, mecamylamine, curare and α-bungarotoxin were ineffective. As compared with other parts of the nervous system where NBT and various antagonists also blocked nicotinic responses, e.g. autonomic ganglia,[24] retinal ganglionic cells,[23] cerebellum,[9] and dorsolateral septum,[53] the pharmacological profile observed in the latter region is the closest to the prefrontal cortex. Indeed, in septal neurons, nicotinic effects were blocked specifically by NBT but not by any other classical nicotinic antagonists. The sensitivity to DHBE differentiates between responses in cortical and septal neurons which otherwise share an unusual feature of the slow time-course of nicotine effects.

The reduction of the eEPSP by muscarinic agonists was less sensitive to pirenzepine than to the other antagonists, atropine or scopolamine, thus suggesting the involvement of M2 receptor subtypes.[11,15] The muscarinic AChRs located on thalamocortical afferents in the anterior cingulate cortex also appear to be of the M2 category.[50]

CONCLUSIONS

Recently, data have accumulated showing that nicotinic drugs improve performances in a variety of cognitive tasks.[22] These effects are observed in Alzheimer patients, in animals with basal forebrain lesions and in intact subjects. The beneficial actions of nicotine appear to be more pronounced in tasks requiring new reversal learning and working memory.[16,29,31,38] These tasks also critically involve the prefrontal cortex[4,12,34] and the mediodorsal thalamus.[41,47] It is tempting to speculate that nicotinic AChRs located on the mediodorsal-prefrontal projection would play a particular role in cognitive processes. Experiments designed to address this question are currently under way.

Although the involvement of ACh in cognitive functions has long been recognized, its functional implication at the level of the neocortex is poorly understood. Our data showing a reciprocal regulation of excitatory synaptic transmission via nicotinic and muscarinic AChRs suggest a neuroanatomical framework by which ACh may control information processing in the prefrontal cortex.

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