The combination of nicotine with the D$_2$ antagonist raclopride or the weak D$_4$ antagonist L-745,870 generates a clozapine-like facilitation of NMDA receptor-mediated neurotransmission in pyramidal cells of the rat medial prefrontal cortex

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Abstract
Clozapine and other atypical, but not typical, antipsychotic drugs (APDs), facilitate both dopaminergic and N-methyl-D-aspartate (NMDA) receptor-mediated glutamatergic transmission in the medial prefrontal cortex (mPFC), which is thought to improve cognition. Switching schizophrenic patients from typical APDs to clozapine may reduce their cigarette smoking. Here, we tested whether nicotine, which facilitates dopamine release, also facilitates NMDA receptor-mediated neurotransmission in the mPFC, when given alone or in combination with a D$_2$ antagonist, raclopride, or a D$_4$ antagonist, 3-(4-[4-chlorophenyl]piperazin-1-yl)methyl-1H-pyrrolo[2,3-b]pyridine (L-745,870), using intracellular recording in pyramidal cells of the rat mPFC. Neither nicotine nor raclopride or L-745,870 alone altered NMDA-induced currents in these cells. However, combining nicotine with raclopride or L-745,870 facilitated these currents. Similarly to clozapine the combination of nicotine with raclopride or L-745,870 also markedly potentiated evoked excitatory post-synaptic potentials in the mPFC. Our results support the idea that intense smoking in schizophrenia may represent a form of self-medication with nicotine.

Received 10 February 2004; Reviewed 7 April 2004; Revised 8 June 2004; Accepted 9 June 2004

Key words: Clozapine, D$_2$ antagonists, nicotine, NMDA receptor, prefrontal cortex.

Introduction
Schizophrenic patients are frequently heavy smokers (Hughes et al., 1986) and it has been suggested that the intense tobacco consumption by schizophrenic patients may represent a form of self-medication with nicotine, and nicotine has been proposed to improve both cognitive dysfunction as well as negative symptoms in schizophrenia (Nomikos et al., 2000; Simosky et al., 2002; Svensson et al., 1990). Interestingly, treatment with the atypical antipsychotic drug (APD) clozapine, but not typical APDs, decreases smoking in schizophrenic patients (McEvoy et al., 1995).

The symptomatology of schizophrenia has, in part, been related to an aberrant glutamatergic neurotransmission in the brain, particularly at the N-methyl-D-aspartate (NMDA) receptor level. This idea is supported by several lines of evidence. Thus, non-competitive NMDA receptor antagonists, such as phencyclidine, may even in healthy volunteers elicit a psychosis that is virtually indistinguishable from schizophrenia, including cognitive, positive, and negative symptoms, typical thought disorder and even auditory hallucinations (see Javitt and Zukin, 1991; Svensson, 2000). Another study showed, for example, alterations in the expression of NR2 subunit mRNA in the prefrontal cortex (PFC) of schizophrenic patients (Akbarian et al., 1996), and, indeed, during the past few years a number of genetic association studies have identified susceptibility genes for schizophrenia, many of which are, in one way or another, linked to the glutamate system.
Clinical evidence indicates that clozapine and some other atypical APDs possess superior efficacy over typical APDs in the treatment of schizophrenia, particularly against cognitive and negative symptoms (see Davis et al., 2003), effects that in part be explained by their ability to markedly enhance prefrontal dopamine (DA) release (Moghadam and Bunney, 1990; Nomikos et al., 1994). Indeed, previous results strongly implicate prefrontal DA in the control of the cognitive functioning, e.g. working memory, an action mediated via DA D<sub>2</sub> receptors (Goldman-Rakic et al., 2000). Interestingly, previous data demonstrate that atypical, but not typical, APDs markedly facilitate NMDA and electrically evoked excitatory postsynaptic potential (EPSP) responses in pyramidal cells of the rat medial prefrontal cortex (mPFC; Arvanov et al., 1997; Arvanov and Wang, 1998). Moreover, the facilitatory effect of clozapine seems to involve a coupling between DA D<sub>2</sub> and NMDA receptors in the mPFC (Chen and Yang, 2002; Ninan and Wang, 2003). Thus, both DA and glutamate may be linked to the action of clozapine in the mPFC.

Our previous data also show that nicotine may increase prefrontal DA release, an effect that appears enhanced with repeated administration (Nisell et al., 1996). Moreover, both electrophysiological and biochemical data support a glutamate-releasing effect of nicotine in the brain, including the PFC (Gioanni et al., 1996). Moreover, both electrophysiological and biochemical data support a glutamate-releasing effect of nicotine in the brain, including the PFC (Gioanni et al., 1996). Against the above background we have, in this paper, investigated the effect of nicotine on NMDA receptors in the mPFC (Campden model MA 752, World Precision Instruments, Sarasota, FL, USA) and kept submerged in Ringer’s solution at room temperature for at least 1 h to allow for recovery. A single slice was then transferred to a recording chamber (32 °C) in which it was held submerged between two nylon nets. The chamber was continuously perfused with Ringer’s solution.

Standard intracellular and single-electrode voltage-clamp recording techniques were used to record pyramidal cells in layers V and VI of the mPFC in slice preparations as described previously (Arvanov et al., 1997; Arvanov and Wang 1998). Briefly, electrodes were pulled by using a horizontal electrode puller (Model P-87; Sutter Instrument Company, San Rafael, CA, USA), filled with 2 M K-acetate (the tip resistance was between 55 and 120 MΩ), and used for recording the mPFC neurons with an Axoclamp 2A (Axon Instruments, Foster City, CA, USA) amplifier. Single-electrode voltage-clamp (holding potential −60 mV) was performed in discontinuous mode with a sampling rate of 5–6.2 kHz. The voltage recordings were acquired using digital-analogue sampling and acquisition software (Clampex 7; Axon Instruments). During the voltage-clamp recordings of NMDA-evoked currents, tetrodotoxin (TTX; 0.5–1 μM, to block the action potentials), glycine (1 μM, to enhance the NMDA-induced responses) and bicuculline [5–10 μM, to block the γ-aminobutyric acid type A (GABA<sub>A</sub>) receptor responses] were routinely included in Ringer’s solution.

The procedures for eliciting EPSPs by electrical stimulation of the forceps minor (white matter) have been described elsewhere (Arvanov et al., 1997; Chen and Yang, 2002). Briefly, electrodes for current-clamp experiments were filled with 2 M K-acetate. Bicuculline, 2–5 μM, was included in the bath solution. EPSPs were elicited by passing rectangular current pulses (0.3 ms duration, 5–20 V) between the tips of a bipolar stainless-steel electrode placed in the medial part of the forceps minor close to the recording electrode (Arvanov and Wang, 1998). A train of six electrical pulses was delivered at a rate of 0.05 Hz, before and every 10 min after drug application. The EPSPs were recorded in the current-clamp mode.

TTX, bicuculline, glycine, clozapine, nicotine, raclopride, L-745,870, and D-(-)-2-amino-5-phosphono-pentanoic acid (D-APV) were administered via bath perfusion of Ringer’s solution. NMDA (5–20 μM) was
pyramidal cells of the mPFC have a relatively long spike duration (1–3 ms at half-maximum spike amplitude) and show a pronounced spike-frequency adaptation in response to constant-current depolarization pulses. In Ringer’s solution, presumed pyramidal cells of layers V and VI in the rat mPFC exhibited a mean resting membrane potential of \(-79 \pm 4\) mV (n = 17 cells), an action potential amplitude of \(101 \pm 6\) mV (n = 16), a spike half-width of \(2.3 \pm 0.2\) ms (n = 16), and an after-hyperpolarization potential of \(7 \pm 1\) mV (n = 16). These results are comparable to those previously published (Arvanov et al., 1997).

As reported previously (Arvanov et al., 1997) bath perfusion with clozapine (100 nM, n = 4) markedly potentiated the NMDA-induced currents (see Figure 1a, b) in pyramidal cells of the mPFC. On the other hand, no effect was seen on the NMDA-induced currents with raclopride (1 \(\mu\)M, n = 6), L-745,870 (100 nM, n = 4), or nicotine (100 nM, n = 4) (Figure 1b). However, perfusion with nicotine + raclopride (n = 4) or nicotine + L-745,870 (n = 4) significantly facilitated the NMDA-induced currents in the mPFC (p < 0.05, one-way ANOVA followed by LSD post-hoc comparison; Figure 1b).

We also examined the effect of clozapine as well as the combinations of nicotine + raclopride and nicotine + L-745,870, on polysynaptically mediated evoked EPSPs in pyramidal cells of the mPFC. Consistent with earlier reports, clozapine (1 \(\mu\)M, n = 5, Figure 2a) markedly evoked bursts of action potentials riding over EPSPs with variable onset latencies (Chen and Yang, 2002). However, when administered alone, nicotine (1 \(\mu\)M, n = 4, Figure 2c), raclopride (1 \(\mu\)M, n = 3, Figure 2b), and L-745,870 (1 \(\mu\)M, n = 3, Figure 2d) did not produce this effect. Both the combinations of nicotine (1 \(\mu\)M) + raclopride (1 \(\mu\)M) and nicotine (1 \(\mu\)M) + L-745,870 (1 \(\mu\)M) mimicked the effect of clozapine on the electrically evoked EPSPs (see Figure 2e, f; n = 5 and 3 respectively). The effects of nicotine + raclopride (n = 3) and nicotine + L-745,870 (n = 3) on the EPSPs were blocked by the NMDA receptor antagonist D-APV (50 \(\mu\)M; see Figure 2g,h).

**Discussion**

The present results demonstrate that whereas raclopride, L-745,870, and nicotine, when given alone, did not potentiate NMDA-induced currents in pyramidal cells in layers V and VI of the mPFC, the combined treatment with nicotine and either of the two DA receptor antagonists caused a clozapine-like potentiation of NMDA receptor-mediated glutamatergic transmission in the mPFC.

**Results**

The electrophysiological criteria for distinguishing presumed pyramidal from non-pyramidal neurons have been described previously (see Arvanov et al., 1997; Arvanov and Wang, 1998). Briefly, the presumed
TTX-insensitive, in analogy with the previously unpublished observations. The potentiating effect of nicotine in combination with a D<sub>4</sub> antagonist D-APV. The EPSPs were recorded in the current-clamp mode.

The facilitating effect of nicotine of action potentials in pyramidal cells of the mPFC. (g, h) The combination of nicotine with raclopride, or nicotine + L-745,870, did not produce any effect on the electrically evoked EPSPs in the mPFC. (e, f) The combination of nicotine with raclopride, or L-745,870, mimicked the effect of clozapine in facilitating electrically evoked EPSPs and bursts of action potentials in pyramidal cells of the mPFC. (b–d) Raclopride, nicotine, and L-745,870, on the EPSPs was blocked by the NMDA receptor antagonist D-APV. The EPSPs were recorded in the current-clamp mode.

The nicotine concentrations in this study correspond to the whole-brain concentrations generated by systemic administration of a nicotine dose of 15 µg/kg. This results in the rat in serum concentrations which correspond almost exactly to the serum concentrations of nicotine in humans 5 min after smoking 1–3 cigarettes. Consequently, the nicotine concentrations employed in this study should represent a clinically relevant concentration range (de Villiers et al., unpublished observations).

The underlying mechanism may, tentatively, involve prefrontal DA. The potentiating effect of the combination of a DA antagonist and nicotine was TTX-insensitive, in analogy with the previously demonstrated, similar effect of clozapine, which is DA dependent and involves activation of D<sub>4</sub> receptors (Ninan and Wang, 2003). Recent evidence indicates that nicotine may influence prefrontal DA release by means of a TTX-insensitive modulation of the DA transporter, an effect which seems mediated via nicotinic acetylcholine receptors (nAChRs) located on the dopaminergic afferents (Drew et al., 2000; Drew and Werling, 2003). Indeed, presynaptically located nAChRs have been proposed to elicit a TTX-insensitive neurotransmitter release in several brain regions (see Wonnacott, 1997). Although, D<sub>2</sub> antagonists, such as haloperidol and raclopride, may cause a slight increase in prefrontal DA release (Moghadam and Bunney, 1990; Nomikos et al., 1994), this effect, which is much smaller than that of clozapine, seems in itself insufficient to modulate glutamatergic neurotransmission, as no such effect was obtained in this as well as in a previous study (Arvanov and Wang, 1998). However, the combination of a D<sub>2</sub> antagonist and nicotine may well generate a larger DA-releasing effect in the mPFC, similar to that of clozapine, and therefore allow for the facilitation of NMDA receptor-mediated neurotransmission.

Figure 2. Representative traces illustrating the effect of nicotine in combination with raclopride or L-745,870 on excitatory post-synaptic potentials (EPSPs), induced by electrical stimulation of the forceps minor, in pyramidal cells of the mPFC. (a) Clozapine-evoked spikes riding over late EPSPs in the pyramidal cells. (b–d) Raclopride, nicotine, and L-745,870, did not produce any effect on the electrically evoked EPSPs in the mPFC. (e, f) The combination of nicotine with raclopride, or L-745,870, mimicked the effect of clozapine in facilitating electrically evoked EPSPs and bursts of action potentials in pyramidal cells of the mPFC. (g, h) The facilitating effect of nicotine + raclopride, or nicotine + L-745,870, on the EPSPs was blocked by the NMDA receptor antagonist D-APV. The EPSPs were recorded in the current-clamp mode.
neurons leading to bursts spikes riding over EPSPs with different onset latencies in the recorded cells. The clozapine-induced potentiation of the EPSPs could be blocked by the NMDA receptor antagonist D-APV, suggesting that the effect of clozapine on the interactions between the pyramidal cells is, indeed, mediated by NMDA-receptors (Chen and Yang, 2002). By inference, our results might indicate that the combination of nicotine and antagonists at DA receptors within the D2 family can mimic the effect of clozapine and also facilitate NMDA receptor-mediated network interactions between interconnected pyramidal cells.

In conclusion, the present results show that the combination of nicotine with a D2- or a weak D4 antagonist can produce a facilitation of NMDA receptor-mediated glutamatergic neurotransmission in the mPFC, similarly to clozapine, suggesting that the addition of nicotine to antagonists at receptors within the D2 family may contribute, at least temporarily, to improving cognitive and negative symptoms in schizophrenia. Accordingly, our data may also have a bearing on the decreased smoking in patients with chronic schizophrenia when their treatment is switched from typical APDs to clozapine.

Acknowledgements

The present study was supported by the Swedish Research Council, grant no. 4747, and the Karolinska Institutet. Dr Jardemark was supported by a NARSAD Young Investigator Award and a grant from the Scandinavian College of Neuro-Psychopharmacology and the Lundbeck Foundation. We thank Dr Björn Schilstrom and Dr Sabina de Villiers for productive discussions.

Statement of Interest

None.

References


