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Effects of nicotine and mecamylamine on rat dorsal raphe neurons

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Abstract

This study investigates the hypothesis that serotonin mediates certain nicotine effects, such as mood improvement and the suppression of the ponto-geniculo-occipital spikes of rapid eye movement sleep. The influence of nicotine (10–300 μM) on the firing rate of dorsal raphe neurons and on serotonin release was therefore, studied in rat midbrain slices. Nicotine increased the firing rate, 10–90%, in 67.5% recorded neurons and decreased it, 8–100%, in the remaining 32.5%. Serotonin release increased 2–7 times after nicotine administration, regardless of firing frequency, but the absolute value of serotonin release was 3 times higher during the decreases than during the increases in firing rate. Mecamylamine (1–20 μM) transiently stimulated the dorsal raphe neurons and competitively antagonized the nicotine-induced serotonin release. The results support the working hypothesis and additionally show that mecamylamine also stimulates dorsal raphe neurons.

Keywords: Nicotine; Dorsal raphe nucleus; Serotonin; Mecamylamine; Brain slices

1. Introduction

Recent results obtained in our laboratory showed that transdermally applied nicotine improves mood (Salín-Pascual and Drucker-Colín, 1998) and suppresses the ponto-geniculo-occipital spikes of rapid eye movement (REM) sleep in cats (Vazquez et al., 1996). These same events appear to be serotonin-dependent, since electrical stimulation of the dorsal raphe nucleus, the largest pool of serotonergic neurons of the brain, suppresses the ponto-geniculo-occipital spikes (Brooks and Bizzi, 1963; Mc Ginty and Harper, 1976; Simon et al., 1973) and blockers of serotonin re-uptake have major antidepressant actions (Wilner, 1985; Blier et al., 1987). Another similarity between nicotine and serotonin actions was observed in the regulation of appetite since both systemic nicotine (Schwid et al., 1992) and serotonin re-uptake blockers (Wurtman and Wurtman, 1979) cause weight loss by decreasing food intake and especially carbohydrate intake.

These serotonin like effects of nicotine may be explained by a nicotine-induced serotonin release, especially in the dorsal raphe nucleus. This hypothesis is supported by the presence of numerous nicotinic receptors on dorsal raphe neurons of mice (Marks et al., 1992), rats (Segal et al., 1978; Deutch et al., 1987), cats (Pin et al., 1968) and humans (Benwell et al., 1988) and by the well-documented role of nicotinic pre-synaptic receptors in facilitating neurotransmitter release (for review see Wonnacott, 1997).

Studies performed in brain slices showed that nicotine releases serotonin in striatum (Westfall et al., 1983) and hypothalamus (Hery et al., 1977), whereas microdyalisis studies demonstrated that systemic nicotine increases serotonin release in the frontal cortex (Ribeiro et al., 1993). However, to this date there are practically no studies concerning the effects of nicotine on dorsal raphe neurons (Li et al., 1998). In the present study, rat midbrain slices were used to examine nicotine's effects on firing rates of dorsal raphe neurons and in serotonin release.

2. Materials and methods

2.1. Brain slice preparation

The experiments were performed in coronal midbrain slices obtained from young male Wistar rats

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(b.w. = 140–160 g), anaesthetized with chloral hydrate (400 mg/kg, intraperitoneally). After decapitation, the brain was rapidly (< 1 min) removed and transferred into a Petri dish, containing ice-cold Yamamoto buffer (composition, in mEq/l: NaCl, 124; KCl, 5; $CaCl_2$, 2.4; KH_2PO_4, 1.24; $MgSO_4$, 1.3; $NaHCO_3$, 26; and glucose, 10). Coronal slices (350 μm width) were obtained using a McIllwain tissue chopper. The slices containing the dorsal raphe nucleus, localized in the region where the aqueduct opens into the fourth ventricle, were transferred in a tissue slice recording chamber (Fine Science Tools) and completely immersed into the flowing perfusate (Yamamoto buffer, 1.5 ml/min, 35.5°C, oxygenated and maintained at a pH of 7.44 by bubbling it with carbogene). Noradrenaline (Arterenol, Sigma) was added to the perfusate up to 50 μM, in order to induce the automatic firing of otherwise silent serotoninergic dorsal raphe neurons (VanderMaelen and Aghajanian, 1983). The area of dorsal raphe nucleus inside each slice was identified in the midline, between the medial longitudinal fasciculi and the aqueduct.

2.2. Recordings

The electrical activity of 72 dorsal raphe nucleus neurons was recorded extracellularly, 1 h after the isolation of the slices, with glass microelectrodes filled with 3 M NaCl and having impedances of 4–8 MΩ. The signals were amplified 500–1000 times, filtered (300–3000 Hz), visualized with an oscilloscope and recorded with a digital tape recorder (48 kHz sampling rate). Rate histograms were obtained off-line, using a Brain Wave system. The serotoninergic neurons were identified using the electrophysiological criteria proposed by VanderMaelen and Aghajanian (1983): slow firing rate (0.5–2.5 Hz), regular rhythm, wide (1.5–3 ms) biphasic action potentials.

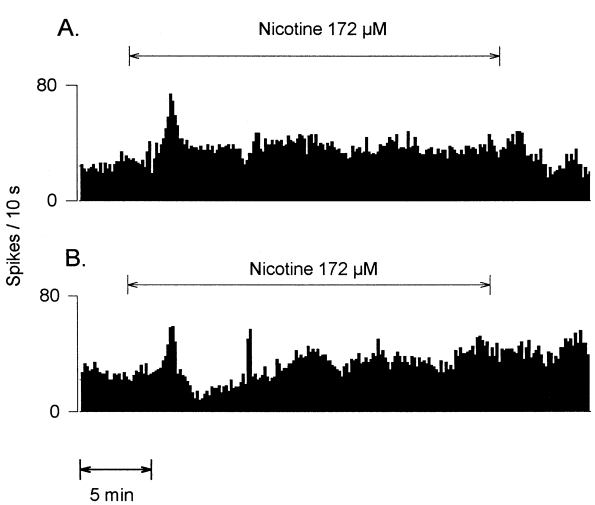

Fig. 1. Oscillatory behavior of the firing rate of two dorsal raphe neurons, induced by long lasting perfusions with nicotine. (A) The beginning of nicotine's stimulatory effect is marked by an increase of the firing rate up to 7 Hz during around 100 s, followed by oscillations with lower amplitudes (around 2 Hz); (B) the initial short (80 s) and high-amplitude (6 Hz) increase in firing rate is followed by a quasi-symmetrical decrease and afterwards by lower amplitude oscillations. Note the increase in firing rate after the interruption of nicotine perfusion.

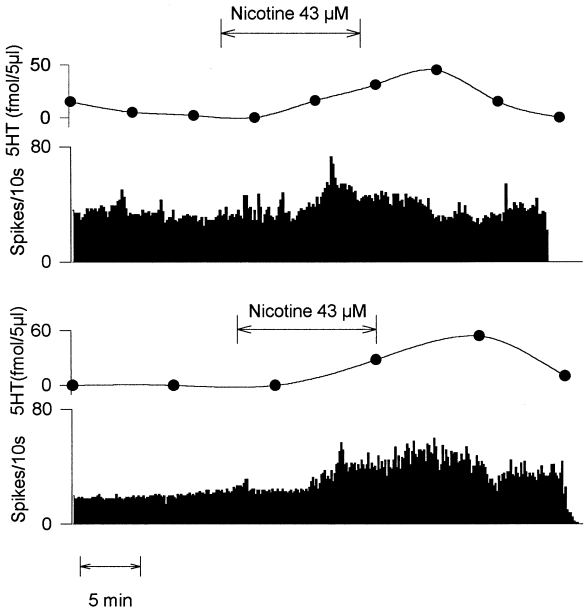

Fig. 2. Stimulatory effects of nicotine on neuronal firing rate and on serotonin release. In both graphs, the peak of serotonin release is reached after the peak of firing rate increase.

2.3. Drugs

Nicotine (hydrogen tartrate salt, Sigma) was administered in perfusion (10–300 μM), using an additional reservoir, coupled with the perfusion line through a three-way stopcock. The perfusions with nicotine lasted 10 min; in five experiments we used long-lasting perfusions with nicotine (25–30 min) in order to study the nicotine-induced oscillatory behavior of the firing rate and serotonin release. Generally, two administrations of nicotine, separated by 10–15 min periods of washout, were used in each experiment.

Mecamylamine (Sigma, 1–20 μM), a non-competitive nicotine receptor blocker, was added to the perfusate from the additional reservoir, and left in contact with the slices 20 min before the administration of nicotine.

2.4. Estimation of serotonin release

Serotonin concentration was measured in samples collected each 5 min from the perfusate's vacuum line. The samples were analyzed by high performance liquid chromatography with electrochemical detection. We used a 100×1 cm^2 column (Bioanalytical Systems, 3 mm) with a 5-μl sample loop. The mobile phase contained: 100 mM monochloracetic acid, sodium octilsulfate 223 mM, 0.5 mM Na_2EDTA, 25 ml acetonitrile 5% and 4 ml tetrahy-

drofuran; the pH was set at 3.1 using NaOH. We used a Petit Amper (Bioanalytical Systems) EC detector model LC3C with a glass carbon electrode vs. Ag/AgCl electrode at 0.65 V. The flow rate was 80 μl/min and the retention time of 15 min. Serotonin was identified using an external standard (fmol/5 μl) and was quantified by measuring the area under the identified peaks.

2.5. Data analysis

All results are expressed as means ± S.E.M. The ED_{50} for nicotine-induced increases of serotonin release was calculated graphically from the dose–response curves. Means were compared by the Student's *t*-test (single comparisons) or by One-way analysis of variance followed by the Bonferoni test (multiple comparisons); the differences were considered significant for $P < 0.05$.

3. Results

3.1. Effects of nicotine on the firing rate of dorsal raphe neurons

The dorsal raphe neurons used for recordings matched the electrophysiological criteria proposed by VanderMaelen and Aghajanian (1983) for identifying serotoninergic neurons: slow firing rate (2.3 ± 0.1 Hz, $n = 72$), regular, wide (1.5–3 ms) biphasic action potentials.

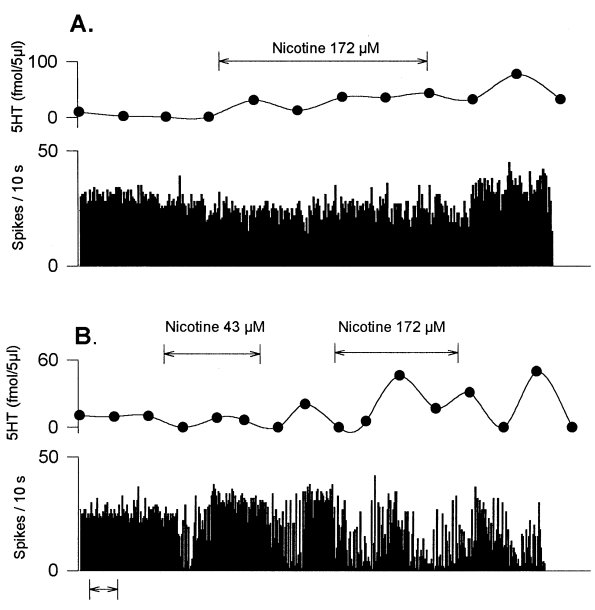

Fig. 3. Inhibitory effects of nicotine on the firing of dorsal raphe neurons associated with increase in serotonin release. (A) The mean serotonin release increases, while the mean neuronal firing rate decreases upon nicotine administration. Note the increase of both parameters upon the interruption of nicotine administration. (B) After the first nicotine administration (43 μM) both the firing rate and serotonin release decrease and then increase, in an oscillatory manner. After the second nicotine administration (172 μM), the mean serotonin release increases, whereas the mean firing rate decreases. The oscillatory increases in serotonin release coincide with decrease of firing firing rate.

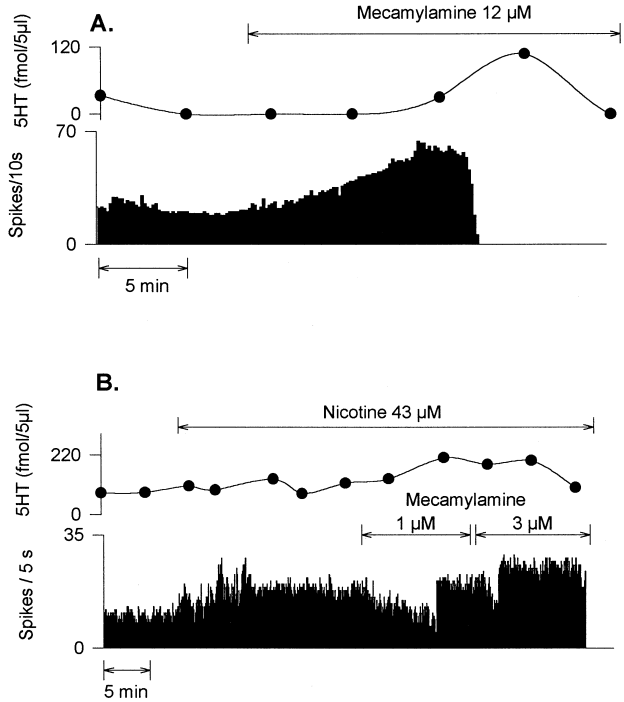

Fig. 4. (A) Increases in firing rate (from 2 to 6 Hz) and serotonin release (from 0 to 150 fmol/5 μl) induced by mecamylamine. There is a sudden ceasing of firing of the dorsal raphe neuron coinciding with the increase in serotonin release. (B) Administered after nicotine 43 μM, mecamylamine (1 and 3 μM) initially reverts the stimulatory effects of this one upon firing rate and serotonin release, but later induces a stimulatory effect on both parameters.

The administration of nicotine produced a sustained increase of the firing rate in 62.5% of the recorded neurons and a decrease in the remaining 37.5%.

The increase in firing rate produced by nicotine (10–300 μM) had a magnitude of 10–90% and was concentration-dependent (Figs. 1, 2 and 5A). The latency of the effect was of 395 ± 18.3 s ($n = 45$) and its duration was proportional to the one of nicotine administration. In 78% of the neurons of this category, the firing rate increase was oscillatory and this was evident during long-lasting (25–30 min) perfusions with nicotine (Fig. 1). The oscillatory response consisted in: (a) an initial 20–30 s increase of the firing rate, up to 6–7 Hz, often followed by a quasi-symmetrical decrease and subsequent oscillations of smaller amplitudes.

Regarding the inhibitory response (Fig. 3), the mean value of the firing rate decrease induced by nicotine was of 57.2 ± 8.4%, with a latency of 205 ± 50 s ($n = 27$). We could not quantify the inhibitory response as being dose-dependent.

In 45% of recorded neurons, with both stimulatory and inhibitory responses to nicotine (Fig. 1B and Fig. 3A),

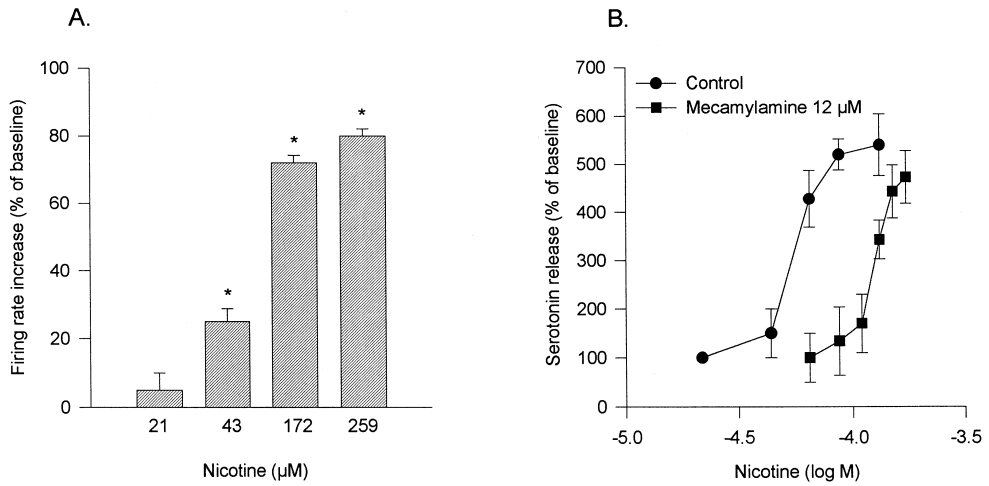

Fig. 5. (A) Dose-dependent stimulating effects of nicotine (43 and 172 μM) on neuronal firing rate. $* P < 0.05$ when compared to baseline firing rate. (B) Mecamylamine shifts to the right the nicotine's dose-dependent curve for serotonin release, without changing significantly its slope or the maximum effect.

there was an obvious increase in firing rate and serotonin release after interrupting nicotine administration.

3.2. Influence of nicotine on serotonin release

Nicotine (10–300 μM) induced a concentration-dependent increase in serotonin release (2–7 times), with an ED_{50} of 60.8 ± 4.3 μM (Fig. 5B). The increase in serotonin release accompanied both the increases and the decreases of neuronal firing rate.

Serotonin release was oscillatory when the neuronal firing rate was also oscillatory. The oscillations of serotonin release and of neuronal firing rate were frequently out of phase, the increases in serotonin release coinciding with decreases in firing rate (Fig. 3B).

When the firing rate increased after the interruption of nicotine administration, serotonin release increased too (Fig. 3A).

The sustained decrease of the mean neuronal firing rate was associated with a sustained increase in serotonin concentration in the perfusate (Fig. 3A and B) up to 89.9 ± 16.9 fmol/5 μl ($n = 15$), value significantly higher than the one observed during the nicotine-induced increase in firing rate, i.e., 26.7 ± 4.8 fmol/5 μl, $n = 25$ (Student's *t*-test, $P < 0.05$). Also, the control values of serotonin release were higher in the neurons responding to nicotine with a decrease in firing rate (16.2 ± 4.3 fmol/5 μl, $n = 15$) than in the neurons responding with an increase in firing rate (4.5 ± 0.7 fmol/5 μl).

3.3. Influence of mecamylamine on the changes in firing rate and serotonin release induced by nicotine

Mecamylamine (1–20 μM) induced a transient (10–15 min) increase of neuronal firing rate by 111–150% ($n = 8$), followed by a return to values close to baseline or, more frequently (75% of recordings), by a disappearance of the spontaneous firing. A 2–3 times transient (5–10 min) increase in serotonin release accompanied the increase of the firing rate of dorsal raphe neurons ($n = 8$). The increase of serotonin release often coincided with the ceasing of the spontaneous firing of dorsal raphe neurons (Fig. 4A). In presence of mecamylamine (12 μM) the nicotine dose–response curves for serotonin release were shifted to the right, without the significant change of the slope or maximum effect (Fig. 5). When administered after nicotine (Fig. 4B), mecamylamine initially reverted the stimulatory effects upon the firing rate and serotonin release, but later significantly increased both parameters up to values higher than the ones produced by nicotine alone, by 6–14% ($P < 0.05$, paired Student's *t*-test, $n = 5$).

4. Discussion

The results of this study confirm the hypothesis that nicotine stimulates the release of serotonin from dorsal raphe neurons. Thus, nicotine (10–300 μM), induced a concentration-dependent increase of serotonin release, by 2–7 times. Therefore, it is conceivable that the suppression of ponto-geniculo-occipital spikes (Vazquez et al., 1996) and the improvement of mood (Salín-Pascual and Drucker-Colín, 1998) represented indirect effects of nicotine, mediated by an increase in serotonin release.

Nicotine administration induced both increases (67.5% of neurons) and decreases (32.5% of neurons) of dorsal raphe neuron firing rates. Similar results were reported by Li et al. (1998), using whole-cell patch in rat midbrain slices. They reported 60% of dorsal raphe neurons responding with depolarization and 40% with hyperpolarization following nicotinic receptor stimulation. These opposing effects on neuronal excitability may be the result of different locations of the recording electrodes in the dorsal raphe nucleus. It is conceivable that direct excitatory and

indirect inhibitory effects of nicotine both occur on the dorsal raphe and play opposite physiological roles.

Nicotine's stimulatory effects on neuronal firing rate may be direct but also indirect. The direct effects of nicotine, mediated by somatic nicotinic receptors, were shown to be predominant in the release of dopamine from the rat striatum (Clarke et al., 1987; Futami et al., 1995). The role of cell body nicotinic receptors on modulation of dorsal raphe neuron excitability and serotonin release has not been yet investigated. In our experiments performed in presence high concentrations of noradrenaline (50 μM), we frequently noticed that nicotine administration induced stable, long-lasting firing of until then silent dorsal raphe neurons. This observation suggests that nicotine may have direct, transmitter-like actions on dorsal raphe neurons, but the confirmation of this supposition requires experiments performed in absence of noradrenaline and in presence of tetrodotoxin (TTX), in order to avoid nicotine's indirect stimulatory effects, as discussed below.

The indirect stimulatory effects of nicotine on dorsal raphe neurons are mediated by the release of glutamate and/or norepinephrine. Thus, Pan and Williams (1989) showed that glutamate mediates the excitatory effects of electrical stimulation of dorsal raphe neurons in rat midbrain slices, whereas McGehee et al. (1995) showed that nicotine, in nanomolar concentrations, releases glutamate in various areas of the brain, process which is TTX-sensitive and hence dependent on stimulation of somatic or preterminal receptors. However, in the study of Li et al. (1998), micromolar concentrations of nicotine induced a slow excitatory postsynaptic potential in dorsal raphe neurons, mediated by a TTX-sensitive and calcium-dependent release of noradrenaline.

The inhibitory effects of nicotine on firing rate are most likely only indirect, through the release of serotonin and possibly, of other inhibitory neurotransmitters. Serotonin release was four times higher during the decreases than during the increases of firing rate and this relatively high concentration of serotonin may have inhibited the firing rate by stimulating the type 1A 5-hydroxytryptamine receptors of serotoninergic neurons. The association between a decrease in firing rate and an increase in serotonin release, also observed by Sprouse et al. (1990), can be explained by the action of nicotine on presynaptic receptors of serotoninergic neurons, which increases the serotonin release in a manner relatively independent of the firing rate. This supposition is supported by the lack of TTX-sensitivity of nicotine-induced serotonin release in dorsal raphe neurons, recently reported by Li et al. (1998). Moreover, in the same study, the nicotine-induced serotonin release was unaffected by methyllycaconitine, a blocker of nicotinic receptors with alfa-7 unit, while the nicotine induced noradrenaline release was abolished by the same blocker.

The variability in the amount of serotonin released by nicotine in our study seems to be dependent on the quality of the slices, since the control values of serotonin concentration were also higher in preparations responding with a decrease in firing rate to nicotine administration. An alternative explanation is that nicotine affected differently serotonin's re-uptake in various experiments. In this respect, it was shown that nicotine increases dopamine uptake in the nucleus accumbens of anesthetized rats (Hart and Ksir, 1996).

The nicotine-induced oscillatory pattern of serotonin release and neuronal firing rate may also result from the above-described serotonin negative feedback, since during these oscillations the increases in serotonin concentration coincided most frequently with decreases of neuronal firing rate. The relatively long periods of firing rate and serotonin release oscillations (3–10 min) result presumably from the experimental setup, in which the flowing perfusate impedes a rapid increase of serotonin concentration in the perfusate surrounding the slices.

The increase of neuronal firing rate and of serotonin release observed after the interruption of nicotine administration suggest that nicotine may induce the release of another inhibitory modulator, besides serotonin. This factor may be γ-aminobutyric acid (GABA), since the dorsal raphe contains an important population of active GABAergic neurons (Pan and Williams, 1989; Pan et al., 1989; Johnson, 1994) and since nicotine induces the release of GABA in other areas of the brain (Yang et al., 1996).

Mecamylamine transiently increased the firing rate and serotonin release from dorsal raphe neurons, effect similar to the one observed by Chen et al. (1994) in neurons of rat dorsal cochlear nucleus. These stimulatory properties of mecamylamine, may explain some of its 'intrinsic' effects, synergic with the ones of nicotine, like lowering of body temperature (Martin et al., 1989) or mood improving after smoking withdrawal (Rose et al., 1994). It is possible that these paradoxical stimulatory properties of mecamylamine result from interactions with other ionotropic receptors involved in the control of neuronal excitability. In this respect, it was shown that mecamylamine is a potent inhibitor of NMDA-induced [^{3}H]noradrenaline release from hippocampal slices (Clarke and Reuben, 1996).

Mecamylamine shifted to the right the nicotine's dose–response curve for serotonin release, without altering significantly its slope or the maximum effect. These manifestations, characteristic for a competitive blocker, apparently contradict the view that mecamylamine is a non-competitive nicotinic receptor blocker. There are, however, numerous reports of competitive antagonistic properties of mecamylamine, especially in the periphery (for review see Martin et al., 1989). More recently, Li et al. (1998) reported that mecamylamine completely abolishes the nicotine-induced norepinephrine release, but only reduces the nicotine-induced serotonin release in dorsal raphe neurons.

Finally, since serotonin release was associated to both increases and decreases of firing rates, it is likely that in

the latter case although the response of the cell itself was inhibitory to nicotine, the effect on the slice as a whole was likely to be excitatory, thus the increase in serotonin, which therefore supports the initial hypothesis of a serotoninergic effect of nicotine.

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