Effect of Nicotine on Neuromuscular Transmission in Mouse Motor Synapses

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Nicotine (10 nM) inhibits rhythmic activity of the neuromuscular synapse in mice. This effect was prevented by α-cobratoxin and apamin. Hence, the effects of nicotine are realized via presynaptic neuronal nicotinic cholinoceptors and Ca^{2+}-activated potassium channels.

Key Words: *neuromuscular synapse; nicotine; acetylcholine receptor; α-cobratoxin; apamin*

Nicotine is an alkaloid of tobacco leaves (*Nicotiana tabacum*); its biological activity is determined by highly selective binding with nicotinic cholinoceptors (nChR) leading to their activation [1,2,8]. The spectrum and mechanisms of the effects of nicotine on the nervous system in tobacco smokers are still an important problem [9]. *In vitro* experiments showed that low doses of nicotine (1-10 nM) corresponding to its level in the interstitial fluid of tobacco smokers [2,3] selectively activate neuronal type nChR typical of ganglionic and central neurons and synapses [8,9]. This mechanism is responsible for numerous acute and chronic neurogenic effects of nicotine [5]. It was accepted for many years that nicotine had no effect on the peripheral motor system in the organism of tobacco smoker, specifically, on motor synapses of skeletal muscles), because these synapses contain muscle-type nChR characterized by two-order lower affinity to nicotine compared to neuronal nChR [2,8]. However, recent studies revealed neuronal nChR in mammalian neuromuscular synapses [6,13]. Possible effects of activation of these receptors (highly sensitive to nicotine) in peripheral synapses are little studied. Here we studied the effects and the mode of action of low doses of nicotine during acute action on the motor synapses in mice.

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MATERIALS AND METHODS

The experiments were carried out on the neuromuscular preparations *m. diaphragma—n. phrenicus* isolated from mice. The mice were narcotized with ether and half of the phrenic muscle was isolated with a fragment of the phrenic nerve. The neuromuscular preparation was dissected according to Barstad, placed in a 3-ml chamber perfused with oxygenated Laily solution (95% O_2 and 5% CO_2) containing (in mM): 135 NaCl, 4 KCl, 0.9 NaH_2PO_4, 2.2 $CaCl_2$, 1 $MgCl_2$, 16.3 $NaHCO_3$, 11 $C_2H_{12}O_6$ (pH 7.2-7.4). The were used to record miniature end-plate potentials (MEPP, at least 100 in each synapse) and evoked end-plate potentials (EPP; at least 50 in each synapse) were recorded using intracellular standard microelectrodes (5-10 MΩ resistance). For recording individual EPP, the phrenic nerve was stimulated with 0.1 msec suprathreshold pulses applied at a rate of 0.3 Hz. Rhythmic activity of synapses was examined with pulses applied at rates of 4, 7, and 50 Hz. The data were sampled with a DigiLine digitizer and processed with Mini-Analysis software (Synaptosoft Co.). Statistical significance was assessed by Student's *t* test (for EPP and MEPP amplitudes) and Mann—Whitney test.

RESULTS

Preliminary experiments showed that nicotine (0.1-1 μM) rapidly decreases to zero the amplitudes of MEPP and EPP due to desensitization of muscle-type nChR on the postsynaptic membrane [8].

Application of nicotine in a lower concentration (10 nM) rapidly (within 2-3 min) decreased MEPP amplitude from 1.2±0.1 mV (control value) to 1.03± 0.07 mV (n=7, p<0.05). During the following 2 h, MEPP amplitude gradually decreased to 60% of the control level (0.68±0.04 mV, n=5, p<0.05). The time of MEPP rise and half-decay (control values 1.32±0.60 and 1.50±0.63 msec, respectively) did not significantly change after application of 10 nM nicotine and were 1.40±0.60 and 1.52±0.33 msec, respectively (n=12, p>0.05).

Nicotine (10 nM) significantly decreased the amplitude of individual EPP from 16.3±0.7 mV in the control to 13.0±2.0 mV (n=14, p<0.01), which was comparable by the dynamics and amplitude to those observed with MEPP. Nicotine produced no significant effect on the quantal Ach release (content): 18.56±0.71 in the control (n=15) and 18.31± 0.91 after application of nicotine (n=23, p>0.05).

Then, we analyzed short trains of EPP consisting of 50 signals evoked at repetition rates of 4, 7, and 50 Hz (Fig. 1). Regular changes in EPP amplitude were observed within the train: the short-term increase in EPP amplitude in the beginning of the train (at the repetition rates of 7 and 50 Hz) was followed by its rapid decrease to a stable (decreased) level which persisted until the end of EPP (plateau phase). The following experiments focused on changes in EPP amplitude during this plateau phase.

In the control, the plateau amplitude in trains evoked at rates of 4, 7, and 50 Hz were 79.6±2.2% (n=16), 82.8±2.9% (n=15), and 88.6±2.1% (n=15) relatively to the amplitude of the first EPP, respectively.

Nicotine (10 nM) decreased the plateau level of EPP train at all repetition rates: by 9% at 4 Hz

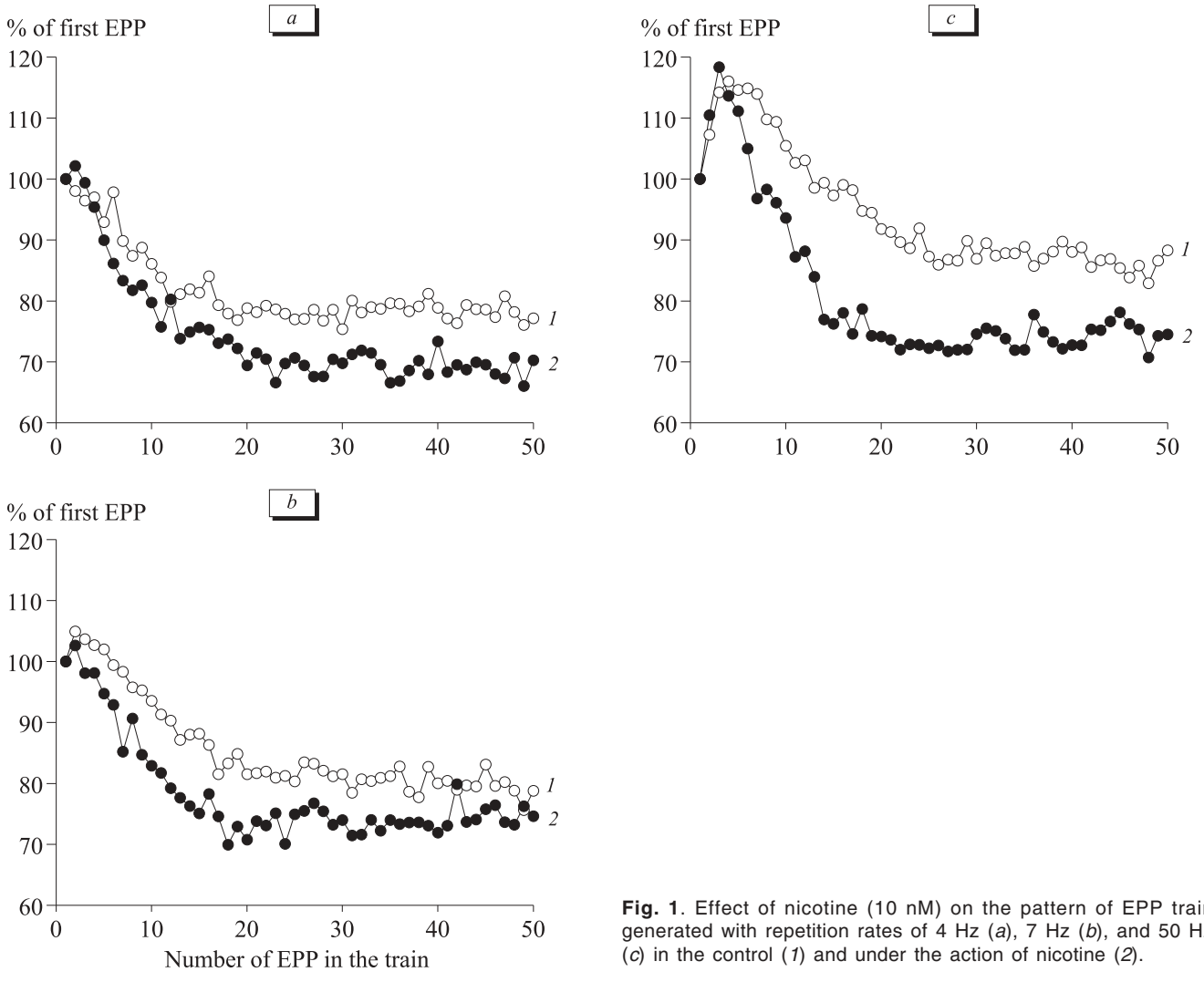

Fig. 1. Effect of nicotine (10 nM) on the pattern of EPP train generated with repetition rates of 4 Hz (*a*), 7 Hz (*b*), and 50 Hz (*c*) in the control (*1*) and under the action of nicotine (*2*).

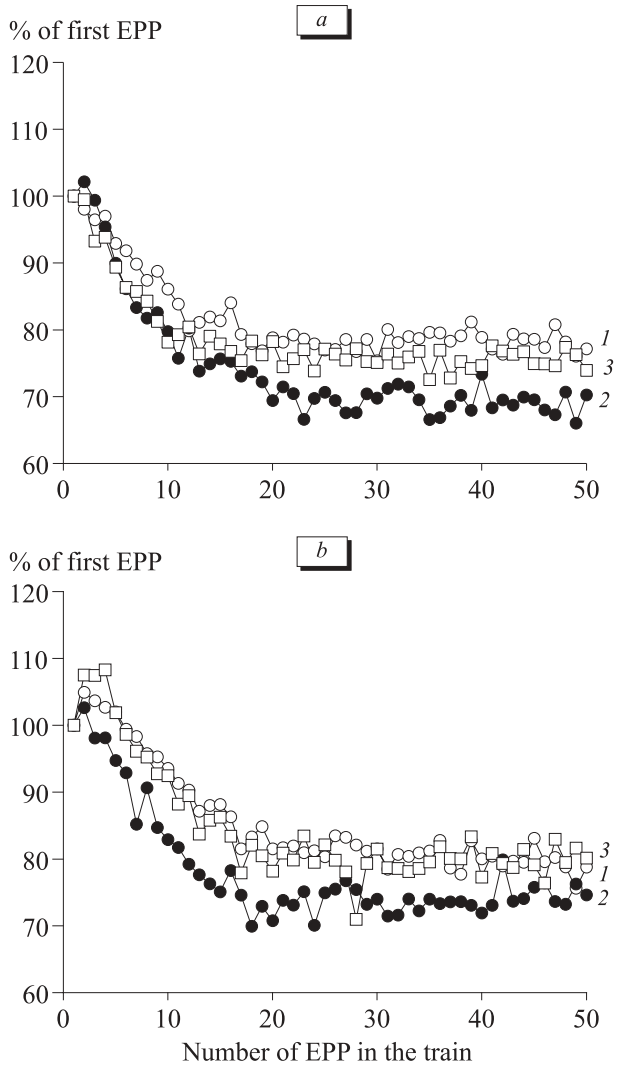

Fig. 2. The envelope curves of EPP trains evoked at the rate of 4 Hz (*a*), 7 Hz (*b*), and 50 Hz (*c*) in control (*1*), or under the action of 10 nM nicotine (*2*) or combination of nicotine and α-cobratoxin (3).

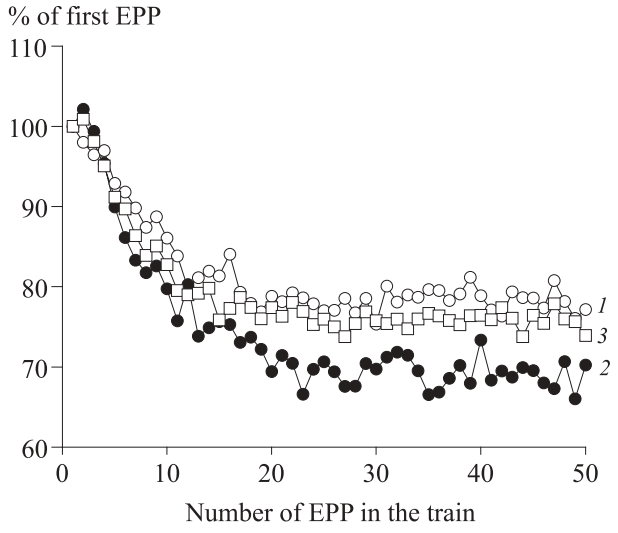

Fig. 3. Effect of apamin (1 μM) on the influence of nicotine on the plateau level of EPP train at repetition rate of 4 Hz. *1*) control; *2*) 10 nM nicotine; *3*) nicotine+apamin.

(to 70.1±2.1% relatively to the amplitude of the first EPP, n=16, p<0.01), by 6% at 7 Hz (to 75.7±2.7%, n=14, p<0.01), and by 8% at 50 Hz (to 79.9±4.0%, n=15, p<0.01).

In mammals (specifically, in humans), the amplitude of EPP in a natural rhythmic burst decreases. The nicotine-induced decrease of EPP amplitude by 8-9% observed in this study during short EPP trains can be physiologically significant in synaptic transmission. Then we studied which types of nChR and mechanisms mediate the established inhibitory effect of nicotine.

Possible involvement of neuronal nChR into the inhibitory effects of nicotine was studied using α-cobratoxin, which selectively blocks neuronal nChR (α7 type included) at nanomolar concentrations [1,2,4,8]. We found that α-cobratoxin (5 nM) produced no significant changes in the amplitude of MEPP, individual EPP, and EPP amplitude in the train. Nicotine (10 nM) applied after α-co-

bratoxin pretreatment had no effect on plateau amplitude of EPP ($p>0.1$, Fig. 2), which indicates possible involvement of neuronal nChR into the inhibitory effect of nicotine.

We previously hypothesized that nicotine can bind nChR in nerve terminals and inhibit evoked acetylcholine (Ach) release via activation of presynaptic apamin-sensitive Ca^{2+}-activated K^+ channels (K_{ca} channels). Similar effect was observed in case of inhibitory action of Ach on firing in some excitable cells [5,10,11,15]. To examine such a possibility in our study, we used apamin (1 μM), a selective blocker of low-conductance K_{ca} channels. This blocker had no effect on train pattern at all tested repetition rates (4, 7, and 50 Hz). Nicotine (10 nM) against the background of apamin (1 μM) had no effect on plateau EPP amplitude, *i.e.* blockade of K_{ca} channels completely prevented the nicotine-induced decrease in plateau EPP amplitude in the train (Fig. 3).

Our experiments showed that long-term (40-120 min) exposure of neuromuscular preparation to nicotine at low concentration (10 nM), which simulates the presence of this alkaloid in interstitial tissue of the tobacco smokers, produces insignificant but stable inhibition of neuromuscular transmission during rhythmic firing. This effect of nicotine is only partially mediated by activation of presynaptic neuronal nChR, because α-cobratoxin prevented only a part of inhibitory effects of nicotine (decrease of plateau in EPP trains).

The potency of nicotine and other cholinergic agonists to inhibit Ach release during rhythmic activity in the peripheral synapses was previously observed during their short-term (1-3 min) application at high (micromolar and higher) concentrations [12, 13]. It was hypothesized that this inhibitory action is mediated via activation of presynaptic neuronal-type nChR. In our study lower concentration of the agonist and longer (1 h) exposure of the neuromuscular preparation with nicotine were used. It is considered that long-term action of agonists desensitize cholinoceptors [4,7]. Were the inhibitory effects of nicotine mediated via desensitization of neuronal-type nChR, they should be reproduced by neuronal nChR blockers. However, we did not observe inhibition of neuromuscular transmission during the action of α-cobratoxin, a blocker of neuronal nChR. At the same time, this toxin prevented the inhibitory effect of nicotine during the plateau phase in EPP train. This phenomenon suggests that despite long-term exposure, nicotine does not completely desensitize neuronal nChR, but exerts its inhibitory effect on Ach secretion via receptors that are selectively blocked with α-cobratoxin. Recent data indicate the possibility of long-term (tens of minutes) action of nicotine on neuronal nChR without pronounced desensitization [4,14].

Activation of neuronal nChR (especially of homomeric α7 type) can induce Ca^{2+} entry into the cell [5,8,9]. Our data also suggest that transport of Ca^{2+} ions into the presynaptic terminal via the channels coupled to neuronal nChR mediates the inhibitory action of the agonist on the burst activity. Ca^{2+} entry can activate apamin-sensitive K_{ca} channels, hyperpolarize the presynaptic terminal, and suppress Ach secretion. There are published data on coupled work of neuronal nChR and apamin-sensitive K_{ca} channels. Their combined action mediates the Ach-induced inhibition of firing in septal neurons [15], hair cells [10], and chromaffin [11] cells.

Location of presynaptic nChR affected by nicotine is unknown. The fact that α-cobratoxin did not facilitate neuromuscular transmission in our experiments suggests that endogenous activators of neuronal nChR (Ach or choline) accumulated in the synaptic cleft do not reproduce the inhibitory effects of nicotine in this mode of synaptic activity. Probably, this phenomenon results from distant location of presynaptic neuronal nChR from the active zones of presynaptic terminal. Being an exogenous agonist, nicotine can reach and activate the pool of nChR, which is inaccessible for endogenous Ach (for example, due to insufficient accumulation of Ach in the synaptic cleft during short-term bursts). The possibility of remote localization of neuronal nChR on the periterminal axonal branches was shown for certain cholinergic synapses in CNS [4].

We established that the long-term inhibitory effect of low nicotine doses on the neuromuscular transmission can be mediated via activation of presynaptic neuronal-type nChR coupled to apamin-sensitive K_{ca} channels.

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