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Variations in desensitization of nicotinic acetylcholine receptors from hippocampus and midbrain dopamine areas

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Abstract

This study addresses two issues arising from the desensitization of nicotinic acetylcholine receptors from the hippocampus, ventral tegmental area, and substantia nigra. First, biophysical studies can find potent and complete desensitization of nicotinic receptors; but in vivo studies often find that desensitization affecting a behavior is less than complete, or that desensitization is important over a different nicotine concentration range. Our results show that there can be significant differences in desensitization when comparing nearby neurons from the same area of the brain. Thus, nicotinic receptors on a minority of neurons may remain active and maintain a behavior under conditions that can produce significant desensitization. Second, agonist applications that are intended to active nicotinic receptors also cause desensitization. The prevailing conditions and the rate of agonist application and removal will control the degree of activation vs. desensitization. These and other factors regulate the efficacy of nicotinic agonists experimentally and physiologically.

Keywords: Nicotine; Activation; Cholinergic; Tobacco; Addiction; Ventral tegmental area

1. Introduction

Desensitized nicotinic acetylcholine receptor channels are closed and refractory to agonist activation. Although desensitization is a complex kinetic process, there are some general principles that serve as guides. Desensitized conformations of the receptor generally have a higher affinity for agonist, and those conformations are achieved more rapidly in high agonist concentrations and are favored by longer exposures to agonist (see reviews Ochoa et al., 1989, 1990; Fenster et al., 1999b).

Nicotinic acetylcholine receptors are pentameric structures often composed of mixtures of α and β subunits (review McGehee and Role, 1995), and both subunit types influence activation and desensitization (Luetje and Patrick, 1991; Cohen et al., 1995; Fenster et al., 1997; Corringer et al., 1998). However, the homomeric α7 receptor shows very rapid desensitization, indicating that both subunit types are not required for the desensitization process. In addition to the genetic makeup of the nicotinic receptor, many other factors modulate the kinetics underlying activation and desensitization, including temperature, voltage, post-translational modifications, various ligands, ionic milieu, and cytoskeletal interactions. In part, the complexities associated with desensitization arise because it is extremely difficult to know and control all the modulatory factors influencing the populations of nicotinic receptors being studied. In this study, we consider two issues that arise from desensitization of nicotinic acetylcholine receptors.

The first issue is the difficulty in explaining in vivo results based on single-cell biophysical studies of nicotinic receptors. When trying to interpret the consequences of nicotine on behavior or on systems-level phenomena, it can be difficult to extrapolate from biophysical results obtained during molecular and cellular studies. Often, nicotine treatments that might be expected to produce nearly complete desensitization based on molecular studies do not completely extinguish a behavior as anticipated. Even after careful consideration of the pharmacodynamics, the equivalent concentration of nicotine that caused nearly complete desensitization in vitro only partially desensitizes when examined in vivo at the systems level. Results from

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this study suggest that this apparent inconsistency arises because there is considerable variability in desensitization of nicotinic receptors even when comparing similar, neighboring neurons under the same experimental conditions.

The second issue we address arises from a problem faced by experimentalists. Experimental attempts to produce more nicotinic-receptor activation by applying higher agonist concentrations are accompanied by faster and more complete desensitization. Extremely rapid agonist applications can synchronize activation, but particularly at high agonist concentrations, that application has to be coupled to rapid removal of agonist to avoid desensitization. At the synapse, rapid applications of high acetylcholine concentrations are achieved via vesicular release into a small cleft, and acetylcholinesterase contributes to the rapid removal of acetylcholine. For this reason, desensitization is not thought to play an important role normally at the neuromuscular junction (Colquhoun and Sakmann, 1998). In the brain where synapses can be closely packed, where firing rates can become high, and where more factors modulate the receptors' kinetics, recovery from desensitization may not always be complete. Furthermore, choline produced by the hydrolysis of acetylcholine may locally achieve concentrations that cause some nicotinic receptor desensitization during high firing rates (see Papke et al., 1996; Alkondon et al., 1997). Results are presented to indicate that a compromise must be struck between activation and desensitization when trying to produce large ongoing nicotinic currents. It also is clear that experimental conditions that produce optimal nicotinic currents under some circumstances will not be optimal under other circumstances. It is likely that the many facets of nicotinic-receptor desensitization that confound experimentalists provide tremendous variability and computational power to the central nervous system.

2. Materials and methods

2.1. Midbrain slices

Midbrain horizontal slices containing the ventral tegmental area and substantial nigra were cut from Sprague–Dawley rats (12–25 days) that had been anesthetized with halothane before decapitation (Pidoplichko et al., 1997). Slices (200–250 μm thick) were cut in ice-cold cutting solution (in mM): 124 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1 $MgSO_4$, 1.25 NaH_2PO_4, 30 $NaHCO_3$, 2 Na-pyruvate, 10 dextrose bubbled with 95% O_2, 5% CO_2. Slices were submerged in a continuously flowing bath solution at 32–34°C of external solution (in mM): 135 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 21 $NaHCO_3$, 10 dextrose, bubbled with 95% O_2 and 5% CO_2, and unless stated otherwise 0.25 to 1 μM atropine was present to inhibit muscarinic acetylcholine receptors. Occasionally 0.5 μM tetrodotoxin was present to inhibit action potentials. The solution in the pipette used for pressure injections contained 1 mM acetylcholine in a bath-like solution except the buffering was with 10 mM HEPES (pH 7.3). Nicotine was applied by bath perfusion in the external solution. Midbrain neurons were identified under infrared light with an upright microscope using Nomarski optics (Zeiss Axioscope, Thronwood, NY).

Patch electrodes were pulled from borosilicate glass (World Precision Instruments, Pensacola, FL) using an upright Kopf model 720 electrode puller (Kopf, Tujunga, CA). The pipette solution was (in mM) 60 $CsCH_3SO_3$, 60 KCH_3SO_3 10 CsCl, 10 EGTA, 5 Mg–ATP, 0.3 Na_3GTP, 10 HEPES, pH 7.3. The holding potential for voltage-clamp recordings was −60 or −70 mV, and the current or voltage measurements were amplified and filtered (0.2–1 kHz) using an Axopatch 1B (Axon Inst.) and were usually digitally sampled at 1–5 kHz. Additional off-line filtering was used for displays.

2.2. Hippocampal cultures

Hippocampal cell cultures were prepared as described previously (Radcliffe and Dani, 1998; Zarei et al., 1999). Sprague–Dawley rats (< 3 days) were anesthetized with halothane and were decapitated. Hippocampi were dissected in ice cold solution containing (in mM) 1.8 $CaCl_2$, 0.81 $MgSO_4$, 5.4 KCl, 140 NaCl, 5.55 D-glucose, 5 HEPES, 0.01–0.1 g/l phenol red, pH 7.3 and were incubated in a sterile enzyme solution (the dissection solution supplemented with 1.5 mM $CaCl_2$, 0.5 mM EDTA, 0.2 mg/ml L-cysteine, 20 units/ml papain) at 37°C in 5% CO_2 for 30 min with gentle rocking. The tissue was washed and dissociated by trituration in minimum essential medium supplemented with 10% fetal bovine serum (HyClone Laboratories,), 10% horse serum (Gibco BRL), 20 mM glucose, 1 μl/ml serum extender (Mito C), 50 units/ml penicillin/streptomycin (Gibco BRL), 2.5 mg/ml trypsin inhibitor, 2.5 mg/ml bovine serum albumin. Cells were plated onto coverslips treated with 0.05 mg/ml poly-D-lysine and 0.25 mg/ml collagen and were kept at 37°C in 5% CO_2. The cultures were fed 2–3 times/week with minimum essential medium supplemented with 5% horse serum, 20 mM glucose, 50 units/ml penicillin/streptomycin, 1 μl/ml serum extender, 10 mM $MgCl_2$, 0–0.5 μM tetrodotoxin, 0–1 nM methyllycaconitine, and 0–0.5% w/v bovine serum albumin and were studied between days 15 and 25, and were treated on days 3–5 with 5 μM cytosine arabinofuroside.

Electrophysiology and solution-exchange techniques were as we have described previously (Radcliffe and Dani, 1998; Zarei et al., 1999). Patch pipettes for patch-clamp recordings were prepared from glass capillary tubing (Garner Glass) and currents were amplified and filtered at 1–2 kHz. Very rapid solution changes to apply agonist were achieved using a linear array of flow pipes (375 μm, inner diameter; Garner Glass) controlled by computer via

valves (General Valve) in the solution pathway. Solutions for the patch-clamp experiments were as follows: external (in mM), 150 NaCl, 0–5 $CaCl_2$, 0–2 $MgCl_2$, 2.5 KCl, 10 Glucose, 10 HEPES, 0–0.2 $CdCl_2$, 0–0.1 picrotoxin, 1 μM tetrodotoxin, 1 μM atropine sulfate, 0–1 μM strychnine, pH7.3, 310–325 mosM; internal solution in the patch pipette (in mM), 140 $CsMeSO_3$, 5 NaCl, 2 Na_2ATP, 2 MgATP, 0–0.3 Na_3GTP, 0.2 EGTA, 10 HEPES, pH 7.3, 300–310 mosM. Picrotoxin (25–100 μM), (±)-2-amino-5-phosphonovaleric acid (AP-5, 50–250 μM), and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) were occasionally used to inhibit synaptic transmission to produce more quiet baselines for recording nicotinic currents. Internal solutions were often supplemented with an ATP regenerating system (20 mM phosphocreatine and 60 units/ml creatine phosphokinase) to decrease rundown of nicotinic currents (Lester and Dani, 1994).

3. Results

3.1. Variable desensitization of nicotinic receptors from the ventral tegmental area

Smokers may experience nicotine concentrations approaching 0.5 μM (Henningfield et al., 1993). Therefore, we bath-applied 0.5 μM nicotine to midbrain slices while inhibiting muscarinic acetylcholine receptors (Pidoplichko et al., 1997). In addition, a pipette containing 1 mM acetylcholine was placed 15–30 μm from the soma to allow brief, local pressure injections of acetylcholine (Fig. 1). The localized puffs of acetylcholine (arrowheads in Fig. 1) produced inward currents mediated by a fraction of the nicotinic receptors located on the soma of the ventral tegmental area neuron. The bath-applied 0.5 μM nicotine initially activated nicotinic receptors causing an inward current observed as a downward shift in the baseline. At much later times, the current decreased and the baseline returned to its original position while nicotine was still present. The result suggests that the great majority of nicotinic receptors were desensitized on this ventral tegmental area neuron under these conditions. This interpretation is reinforced because the acetylcholine-induced currents also became much smaller (arrowheads in Fig. 1).

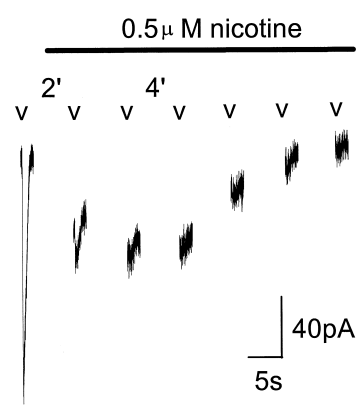

Fig. 1. Strong desensitization of nicotinic receptors was observed in a dopaminergic neuron from the ventral tegmental area upon bath application of 0.5 μM nicotine (solid bar). Local pressure injections of acetylcholine (1 mM for 40 ms, arrowheads) induced inward currents that were desensitized by bath application of nicotine. The injections of acetylcholine were always given every 20 s, but the time interval between displayed traces is initially 2 min, then 4 min. The nicotinic current activated by bath application of nicotine is displayed as a downward displacement of the currents induced by injection of acetylcholine. At later times in nicotine, the baseline current desensitizes and returns toward its original value. The data are adapted from Pidoplichko et al. (1997).

The strong desensitization shown in Fig. 1 was not always observed; rather, the effect of 0.5 μM nicotine was extremely variable. While in current-clamp mode, which more closely approximates the biological situation, bath application of 0.5 μM nicotine initially caused a depolarization of the resting membrane potential and increased the rate of action potentials (Fig. 2). The excitation caused by nicotine depends on a number of factors, including the original resting potential, the threshold for firing action potentials, as well as the number and efficacy of nicotinic receptors. Likewise, the level of desensitization observed at later times also depends on properties of the cell and the nicotinic receptors. Thus, variable effects could be observed, and two very different examples are depicted in Fig. 2. In Fig. 2A, there is some excitation when nicotine initially arrives, but after only a few minutes the neuron ceases firing action potentials even though nicotine is still present. On the contrary, a different ventral tegmental area neuron responded intensely to 0.5 μM nicotine, firing action potentials at a high rate (Fig. 2B). Furthermore, the rapid rate of firing continued for more than 15 min.

3.2. Activation vs. desensitization of nicotinic receptors on hippocampal neurons

In cell culture, rat hippocampal neurons express three types of nicotinic currents (Alkondon and Albuquerque, 1993). The predominant current by far, however, is Type IA current, which is rapidly activating and desensitizing and is sensitive to α-bungarotoxin and methyllycaconitine (Zarei et al., 1999). This pharmacology indicates that the Type IA current is mediated by α7-containing nicotinic receptors (Alkondon et al., 1994; Zarei et al., 1999). That interpretation was verified because Type IA currents are absent from hippocampal neurons derived from α7-null mutant mice (Orr-Urtreger et al., 1997).

Because α7-containing nicotinic receptors desensitize rapidly, it is difficult to pass much charge through the briefly open channels. To optimize the amount of charge entering in a minimum of time via α7-containing nicotinic receptors, we investigated the time-course of recovery from rapid desensitization after a brief nicotine application. A high concentration of nicotine (500 μM) was applied to

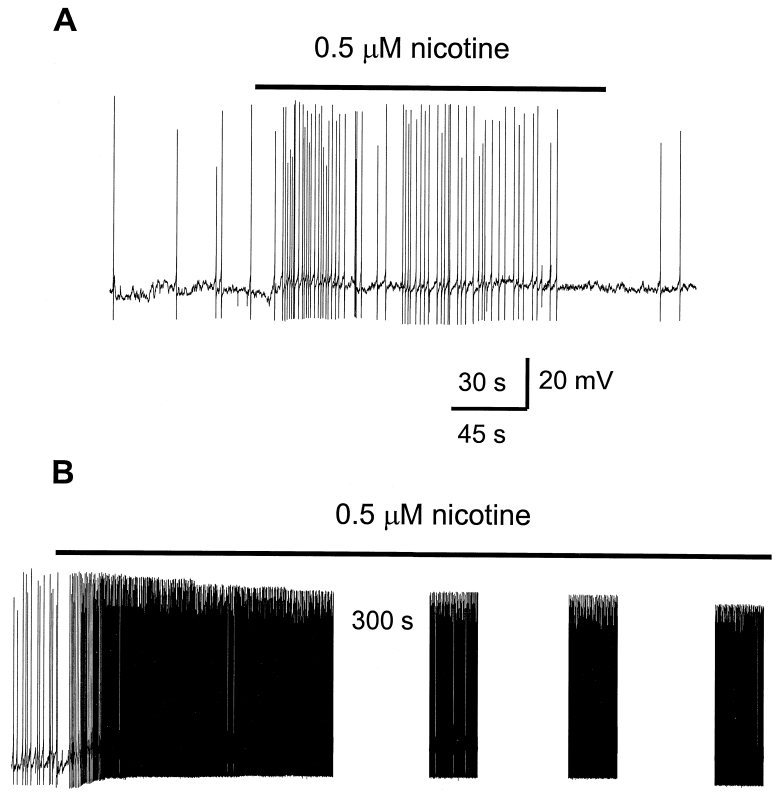

Fig. 2. Desensitization of nicotinic receptors was variable. Current-clamp records are shown from two different ventral tegmental area neurons responding to bath application of 0.5 μM nicotine. The scale bar applies to both figures, with a time scale of 30 s above and 45 s below. (A) Upon arrival of nicotine, the firing of action potentials increases, but the firing ceases while nicotine is still present. (B) Upon arrival of nicotine, the firing rate is increased much more strongly; and it continues while nicotine is present for more than 15 min. The gaps in the recording all represent 300 s.

the whole surface of a cultured hippocampal neuron for a brief time (200 ms, solid bar in Fig. 3A). Then, we waited for 1–30 s before the second application of 500 μM nicotine. After an interpulse interval of 1 s, there was practically no recovery from desensitization, but after 30 s there was complete recovery. The time course of recovery from desensitization is shown in Fig. 3B. Slower rates of recovery would be expected during longer exposures to nicotinic agonists (Lester and Dani, 1994; Reitstetter et al., 1999).

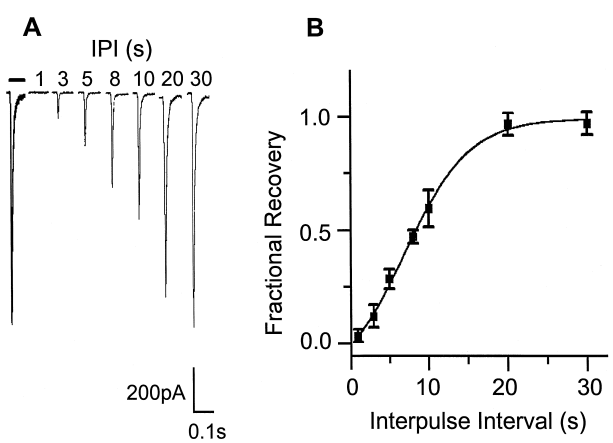

Fig. 3. Recovery from rapidly induced desensitization is shown. Nicotine (500 μM, 200 ms, solid bar) was applied to a cultured hippocampal neuron, inducing rapid activation and desensitization of nicotinic receptors. (A) Repeated runs are shown after waiting for interpulse intervals (IPI) of 1–30 s (as labeled) before the second application of 500 μM nicotine. (B) The time course of recovery from rapid desensitization is shown as a fraction of the current induced by the second nicotine application divided by the current induced by the first nicotine application. The holding potential was −70 mV. The number of trials per data point is three to five, and the S.E.M. are shown.

Fig. 4 shows the consequences of repeated nicotine applications at different interpulse intervals. With an interpulse interval of 10 s or less, the current activated by 500 μM nicotine decreased dramatically with the second application but, then, tended to level off at that smaller amplitude (Fig. 4A). The average data are plotted in Fig. 4B, showing that there was nearly complete recovery from desensitization when the interpulse interval was 30 s. Fig. 5 compares the current activated by the first nicotine application to the current activated by the fifth nicotine application at different interpulse intervals. If the currents are integrated and the charge (Q) passed by the first application is normalized to 100%, the charge passed by the fifth application falls to 10% with an interpulse interval of 3 s and to 88% with an interpulse interval of 30 s (Fig. 5).

If we wish to optimize the charge passed per unit time to accentuate the influence of the nicotinic currents, which

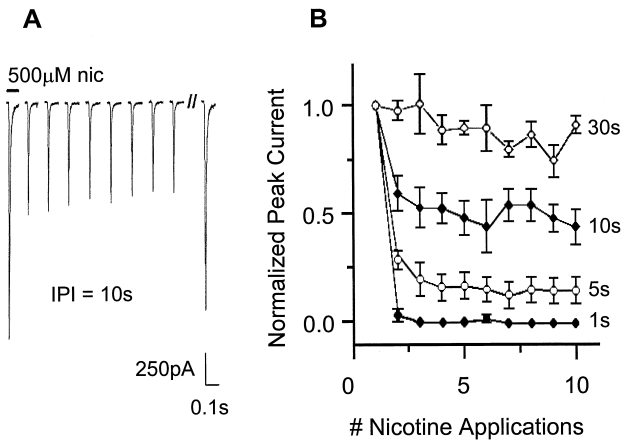

Fig. 4. The response to nicotine (500 μM, 200 ms) at different interpulse intervals (IPIs) is shown. (A) Nicotine-induced currents are shown for an interpulse interval of 10 s. The final current (after the double slash) shows recovery from desensitization after washing for 2.5 min. (B) A series of 10 nicotine applications is shown at different interpulse intervals. For each series, the currents are normalized to the first current. The interpulse interval is given in seconds to the right of each plot. The number of trials is four or five, and the S.E.M. are shown.

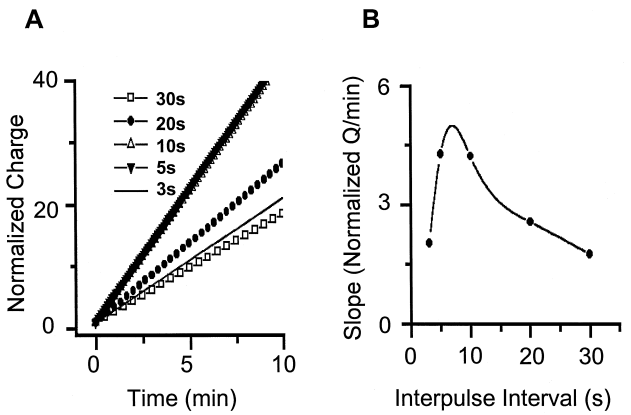

Fig. 6. To maximize the current passed by the nicotinic receptors per unit time, it is not necessarily optimal to wait for complete recovery from desensitization. The charge entry during each of a series of nicotine applications (500 μM, 200 ms) was normalized by the charge evoked by the first application. (A) Considering the charge and the interpulse interval, a plot was constructed of the normalized charge vs. time. (B) A smooth curve was drawn though the slopes of the lines in (A). The data points for the plot were obtained at each interpulse interval that was studied. The maximum charge per unit time under our experimental conditions was estimated to be just over 8 s.

interpulse interval is best? Surprisingly, choosing an interpulse interval of 30 s, which would give the most recovery, does not maximize the charge that enters the cell per unit time over the time range of several minutes (Fig. 6). The optimal interpulse interval under our experimental conditions was about 8 s (Fig. 6B). It is more effective to give multiple applications of acetylcholine that activate only a fraction of the channels each time; rather than waiting and giving no applications until all the channels recover from desensitization. The optimum value we found is very dependent on the initial condition of the cell, the nicotinic-receptor subtypes, and the experimental conditions. For example, we found differences after changing just one experimental parameter, the external Ca^{2+} concentration. Using the same neuron and interpulse interval (8.5 s), the second nicotine application elicited a much smaller current in nominally Ca^{2+}-free solution than in a solution containing 5 mM Ca^{2+}. That finding suggests that a different interpulse interval is required for recovery from desensitization in the Ca^{2+}-free solutions, but other aspects (such as rundown of currents) have to be considered (Lester and Dani, 1994).

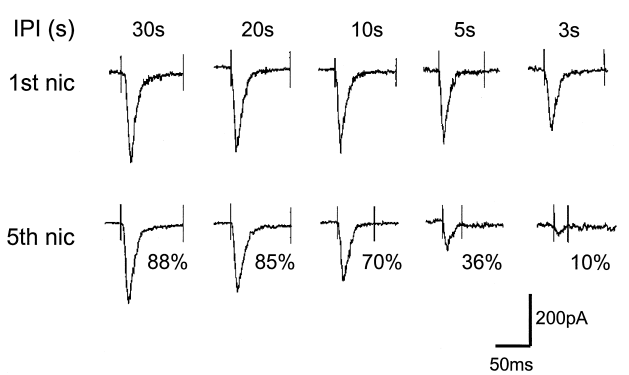

Fig. 5. A comparison of the first and fifth nicotine application (500 μM, 200 ms) in the series at different interpulse intervals is shown. The amount of charge passing through the opened channels was calculated by integrating the area under the currents, and the time intervals for the integrations are indicated by vertical lines across the trances. The charge during the first nicotine application was normalized to 100%, and the relative charge obtained from the fifth application depended on the interpulse interval: 10% with an interpulse interval = 3 s, 36% with an interpulse interval = 5 s, 70% with an interpulse interval = 10 s, 85% with an interpulse interval = 20 s, 88% with an interpulse interval = 30 s.

4. Discussion

4.1. Desensitization of nicotinic receptors varies temporally and spatially

Most nicotinic currents in the ventral tegmental area and substantia nigra arise form standard neuronal nicotinic acetylcholine receptors containing the β2 subunit in combination with α subunits (Picciotto et al., 1998), but there is a minor component of the current mediated by α7-containing nicotinic receptors (Pidoplichko et al., 1997). In the hippocampus, the situation is different, the predominant nicotinic current is mediated by α7-containing nicotinic receptors (Alkondon et al., 1994, 1999; Gray et al., 1996;

Jones and Yakel, 1997; Frazier et al., 1998a,b; Zarei et al., 1999). In support of previous results, we find that the subunit composition strongly influences the kinetics of gating underlying the activation and desensitization processes (Luetje and Patrick, 1991; Cohen et al., 1995; Fenster et al., 1997; Corringer et al., 1998). In addition, those processes are variable in time and location in the central nervous system. When comparing nearby neurons from the same brain slice, the level of activation and desensitization induced by low levels of nicotine can vary greatly. While many nicotinic receptors may be desensitized, some nicotinic receptors will be less susceptible, enabling a minority of neurons to continue responding to nicotine well after most neurons are refractory. Thus, the impact of nicotine as obtained from tobacco may have diverse effects even within one area of the brain, and a wider range of influences is likely when considering multiple areas.

Another issue to be considered is the dynamic nature of a receptor's kinetics. As we have found, simply changing the external Ca^{2+} concentration rapidly alters the desensitization properties of some nicotinic receptors. External Ca^{2+} changes that are in the physiological range also alter the amplitudes of nicotinic currents (Mulle et al., 1992; Vernino et al., 1992; Amador and Dani, 1995). Many other ongoing processes in the cell regulate the kinetics. For instance, intracellular Ca^{2+} and the levels and specific locations of phosphorylation can regulate desensitization of various nicotinic-receptor subtypes differently (Vijayaraghavan et al., 1990; Paradiso and Brehm, 1998; Pardi and Margiotta, 1999). Thus, the kinetics underlying activation and desensitization are dynamically modulated, and the modulation is specific depending on the subunit composition of the nicotinic receptors. It is likely that the variability in desensitization from neuron to neuron and the dynamics of nicotinic-receptor desensitization contribute to the inconsistencies that are sometimes observed when analyzing system-level phenomena based on cellular data.

4.2. Activation vs. desensitization

At a synapse about 1 mM acetylcholine arrives abruptly, nearly synchronously activates nicotinic receptors, and is hydrolyzed by acetylcholinesterase and/or diffuses away. Because the delivery and removal of acetylcholine is very rapid, desensitization is usually not considered to be important at the neuromuscular junction. It should be appreciated, however, that the desensitization process is extremely complex. Muscle nicotinic acetylcholine receptors have been studied in greater detail, revealing multiple rates for entry into and exit out of desensitization (Dilger and Liu, 1992; Franke et al., 1992). Some of the fastest rates suggest there are situations where desensitization is important, as is seen in some disease conditions such as slow-channel myasthenic syndrome (Milone et al., 1997).

Because neuronal nicotinic receptors are diverse and neuronal synapses are anatomically and compositionally complex and varied, the role of desensitization in the brain is a more difficult and important problem. This expectation is particularly true when we consider the low, desensitizing levels of nicotine that bathe the brains of smokers (Ochoa et al., 1990; Clarke, 1991; Dani and Heinemann, 1996). Clearly, those levels of nicotine will desensitize many nicotinic receptors, but not in a uniform or invariant manner. We can expect that extremely active nicotinic synapses will be more susceptible to the desensitizing influence of a smoker's nicotine. Active synapses will be repeatedly exposed to about 1 mM acetylcholine. Normally, that situation might not produce desensitization. If the synaptic stimulation is extremely high, however, even the fast rates of recovery from desensitization may not allow complete recovery, and local accumulation of choline might further contribute to the desensitization of α7-containing nicotinic receptors (see Papke et al., 1996; Alkondon et al., 1997). When those events are occurring in conjunction with hours of exposure to low levels of nicotine, then we can expect those active synapses to be especially susceptible. Evidence indicates that longer exposures to agonist allow slower rates of desensitization to come into play, such that nicotinic receptors can enter longer-lasting desensitization (Lester and Dani, 1994; Reitstetter et al., 1999).

The results presented here also indicate that when considering repeated applications of agonist, waiting for complete recovery from desensitization may not be the optimal way to achieve the maximum charge transfer per unit time. Furthermore, the optimal interval in between agonist applications depends on the overall conditions. These conclusions not only hold for exogenous agonist applications by experimentalists, but also may be significant in the central nervous system. If desensitization comes into play, then the rate of synaptic firing will be an important parameter in determining how much current enters the synapse via nicotinic receptors. The fastest synaptic firing rates would not necessarily produce the most efficacious nicotinic-receptor signal, especially when nicotine from smoking increases desensitization. Furthermore, the subunit composition of the nicotinic receptors sets certain desensitization parameters, and modulatory processes affect the receptor subtypes differently and selectively (Paradiso and Brehm, 1998; Fenster et al., 1999a). Depending on dynamic modulatory influences, different nicotinic-receptor subtypes might become particularly susceptible to desensitization, and these modulatory processes can vary from synapse to synapse. Thus, the same train of action potentials arriving at different synaptic terminals of the same axon could selectively be at the optimal frequency for one nicotinic-receptor subtype while only producing minimal current via another subtype. The most effective nicotinic-receptor subtype at one synapse might not be as effective at a nearby synapse receiving the same train of excitation. In this way,

the Ca^{2+} signal and strength of depolarization could vary greatly even while the nicotinic acetylcholine receptor populations remain constant. These types of processes would provide a continually varying, powerful computational mechanism for manipulating information at synapses.

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