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Nicotinic Acetylcholine Currents in Cultured Postnatal Rat Hippocampal Neurons

CHARLES F. ZORUMSKI, LIU LIN THIO, KEITH E. ISENBERG, and DAVID B. CLIFFORD

Departments of Psychiatry (C.F.Z., L.L.T., K.E.I.), Anatomy and Neurobiology (C.F.Z., L.L.T.), and Neurology (D.B.C.), Washington University School of Medicine, St. Louis, Missouri 63110

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SUMMARY

Nicotinic acetylcholine (ACh) currents were studied in cultured postnatal rat hippocampal neurons, using whole-cell voltage-clamp techniques. In most cells, ACh produces one of two types of response. One class of ACh currents exhibits rapid and profound desensitization and is sensitive to inhibition by α-bungarotoxin (αBTXN). The second class activates slowly and exhibits no desensitization during prolonged agonist applications. This slow current is insensitive to αBTXN. Both the fast and slow responses exhibit inwardly rectifying current-voltage relationships and pass little current at positive membrane potentials. Both currents can be recorded in the presence of 1 μM atropine but are blocked by 0.1–1.0 mM *d*-tubocurarine and 0.1–1.0 mM mecamylamine. These observations suggest heterogeneity of nicotinic ACh receptors in rat hippocampal neurons and provide support for functional αBTXN-sensitive nicotinic receptors in this region.

nAChRs are found in a variety of regions of the vertebrate CNS. These receptors can be divided into two distinct classes, based on affinity for nicotinic ligands. Nicotine and ACh, in the presence of the muscarinic receptor antagonist atropine, appear to label the same high affinity site. In contrast, αBTXN binds to a distinct and differentially distributed population of receptors (1). In most regions of the CNS, reported physiological responses to nicotinic agonists are insensitive to αBTXN (2, 3), suggesting that the toxin binding sites may not be functional receptors. In contrast, nicotine-labeled sites appear to constitute ligand-gated ion channels and participate in synaptic responses in certain regions (3).

Both classes of nicotinic sites are present in the rodent hippocampus, with the density of αBTXN binding sites greatly exceeding that of high affinity nicotine binding sites (1). Furthermore, *in situ* hybridization studies indicate that the hippocampus contains mRNA for multiple nAChR subunits (4–6) and an αBTXN-binding protein (7). The physiology of hippocampal nAChRs is less clear. Disinhibitory responses have been reported, suggesting that the hippocampal nicotinic binding sites mediate physiological effects. *In vivo* recordings have demonstrated that nicotinic agonists augment extracellularly recorded excitatory postsynaptic potentials and population spikes and depress paired pulse inhibition (8, 9). These actions typically have a slow onset and are inhibited by curare and DHBE but not by αBTXN. In hippocampal slices, nicotinic agonists increase the number and amplitude of CA1 population spikes (10). These effects are inhibited by mecamylamine but not by hexamethonium, dTC, or αBTXN (10). Other evidence, although less direct, suggests a physiological role for the hippocampal αBTXN binding site. In slice preparations, αBTXN produces a transient excitation followed by inhibition, leading to an eventual loss of field potentials (10). Additionally, nicotine produces seizures in certain strains of mice (11), and the sensitivity to these seizures is correlated with hippocampal αBTXN binding.

To date, little information is available about the cellular physiology of hippocampal nAChRs. Macroscopic currents (12) and single-channel events (13) evoked by nicotinic agonists have been recorded in cultured embryonic rat hippocampal neurons. These responses are inhibited by dTC, mecamylamine, and DHBE but not by atropine, suggesting that they are mediated by nAChRs. Additionally, the macroscopic currents are inhibited by an α-neurotoxin from *Naja naja kauothia* (12). To develop a better understanding of the physiological significance of nAChRs in the hippocampus, we have characterized nicotinic responses of cultured postnatal rat hippocampal neurons, using whole-cell voltage-clamp techniques and rapid agonist administrations.

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ABBREVIATIONS: nAChR, nicotinic acetylcholine receptor; ACh, acetylcholine; αBTXN, α-bungarotoxin; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; dTC, *d*-tubocurarine; DMPP, 1,1-dimethyl-4-phenylpiperazinium; CNS, central nervous system; DHBE, dihydro-β-erythroidine.

Materials and Methods

Hippocampal cell culture. Hippocampal cell cultures were prepared using established methods (14). Hippocampi were dissected from 1–2-day-old albino rat pups, sliced transversely into approximately 400-μm thick sections, and placed in oxygenated Leibovitz L-15 medium containing 0.2 mg/ml fatty acid-free bovine serum albumin and 1 mg/ml papain (type IV; Sigma Chemical Co.), at 35°. After 30 min in the papain solution, neurons were dissociated by gentle trituration in Eagle's minimal essential medium containing 5% (v/v) fetal calf serum, 5% (v/v) horse serum, 400 μM glutamine, 50 μg/ml streptomycin, 50 units/ml penicillin, and 17 mM glucose. The cell suspension, which contains a mixture of CA1 and CA3 pyramidal neurons, interneurons, and glia, was plated in collagen-coated tissue culture dishes, at a density of 300,000 cells/dish, and incubated at 36° in a humidified 5% CO_2 atmosphere. Cytosine arabinoside (10 μM) was added 48 hr after plating, to inhibit glial proliferation. All media, sera, and antibiotics were purchased from GIBCO (Grand Island NY).

Electrophysiology. Voltage-clamp recordings were obtained using the whole-cell patch-clamp technique (15). For recording purposes, the growth medium was exchanged for a solution containing (in mM) 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES, and 0.001 tetrodotoxin (pH 7.3). Tetrodotoxin was used to diminish spontaneous synaptic activity and to improve the spatial voltage-clamp. Recording electrodes were pulled from fiber-filled borosilicate glass capillaries on a Brown-Flaming P-87 pipette puller (Sutter Instruments). These pipettes were fire-polished to obtain 5–8 MΩ recording electrodes. Pipette solutions routinely contained (in mM) 140 cesium methanesulfonate, 4 NaCl, 5 EGTA, 0.5 $CaCl_2$, and 10 HEPES (pH 7.3). In some experiments, the cesium methanesulfonate was substituted with 140 mM CsCl, 140 mM cesium acetate, or 140 mM sodium isethionate. Similar results were obtained with all intracellular solutions. All recordings were done at room temperature (22°). Cesium methanesulfonate was purchased from Aldrich Chemical Co.; other salts were obtained from Sigma Chemical Co.

Cholinergic agonists and antagonists were dissolved in the recording solution and were applied to neurons using one of two drug delivery systems. In most experiments, drugs were administered from flow tubes with 300-μm openings, positioned about 250 μm from the recorded neuron by using a Narishige hydraulic micromanipulator (16). This system allows complete solution exchange in the vicinity of neurons in 6 ± 2 msec, as judged by the time constant of the rising phase of currents induced by the nondesensitizing glutamate analogue kainate (n = 22). In all experiments using flow-tube drug administrations, the bath was continuously perfused with extracellular solution at a rate of 2 ml/min. In other experiments, drugs were administered by close-range pressure ejection (15–20 psi) from glass pipettes (1–2-μm tip openings) positioned <5 μm from the cell soma (17). Similar results were obtained using either drug delivery system, although the rates of desensitization reported were faster using the flow-tube system, reflecting the more rapid and complete solution exchange in the vicinity of neurons.

Data acquisition and analysis. Whole-cell currents were recorded using a List EPC-7 or Axopatch-1D patch-clamp amplifier. Signals were filtered at 2 kHz and recorded on a Gould 220 chart recorder. In addition, currents were digitized using pCLAMP, version 5.5 (Axon Instruments), and were stored on disk for off-line analysis. In some analyses, the current decay was fit to an exponential function using a least-squares minimization or Gauss-Newton algorithm. Desensitization was measured as percentage of decline:

$$\% \text{ Decline} = \frac{I_{max} - I_{ss}}{I_{max} - I_0} \times 100$$

where I_0 is the base-line current at the holding potential, I_{max} is the peak observed current, and I_{ss} is the observed steady state current measured at the end of an agonist application. All values reported are mean ± standard error.

Results

ACh activates two classes of responses in hippocampal neurons. Rapid flow-tube applications of ACh or other nicotinic agonists to voltage-clamped postnatal rat hippocampal neurons elicited inward currents in 75% (n = 175) of neurons studied between days 5 and 20 after plating. Evoked currents ranged from <100 to 2000 pA, with most cells exhibiting responses in the range of 50–500 pA. The presence or absence of nicotinic responses and the magnitude of the responses did not appear to correlate with either cell morphology or time in culture. The responses were usually one of two types (Fig. 1). In 63% of neurons (62 of 98 cells studied), 100–500-msec applications of 1 mM ACh evoked a rapidly desensitizing current. At −70 mV, these currents decayed monoexponentially, by 91 ± 2%, with a time constant of 8 ± 2 msec (n = 13). Both the rate and degree of desensitization increased with increasing ACh concentration, but neither was dependent on membrane voltage over the range of −90 to −20 mV (Table 1).

In some neurons a distinctly different response to 1 mM ACh was seen (Fig. 1B). These currents activated slowly and showed no evidence of desensitization during agonist applications as long as 10 sec. Slow ACh responses occurred in 23% of neurons (23 of 98 cells studied). In a small number of cells (4 of 98), 1 mM ACh elicited both a rapidly desensitizing response and a more slowly rising, persistent response (Fig. 1C). The amplitude

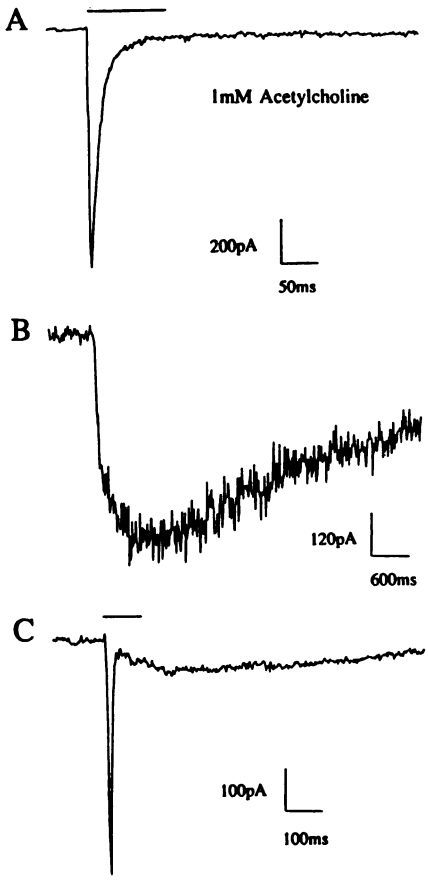

Fig. 1. ACh gates two types of current in cultured hippocampal neurons. The traces depict the responses of three neurons, voltage-clamped at −70 mV, to 100-msec flow-tube applications of 1 mM ACh. The traces show neurons exhibiting the fast desensitizing current (A), the slow response (B), and both responses (C). In this and all subsequent figures, the *bars above the data traces* denote the period of drug application.

TABLE 1

Nicotinic agonists and desensitization

Neurons were voltage-clamped at various membrane potentials and exposed to 100–200-msec flow-tube applications of agonists at the concentrations specified. $\tau_{desensitization}$ was measured from exponential fits to the decaying phase of currents during the application of agonists. Percentage of decline was measured as described in Materials and Methods. V_H is the membrane holding potential. Results are mean ± standard error.

Agonist	Concentration	n	V_H	$\tau_{desensitization}$	Decline
	mM		mV	msec	%
ACh	1	8	−90	8 ± 1	93 ± 1
ACh	1	13	−70	8 ± 2	91 ± 2
ACh	1	7	−20	8 ± 1	84 ± 6
ACh	0.5	8	−70	12 ± 1	76 ± 6
ACh	0.1	5	−70	41 ± 10	49 ± 10
ACh	10	5	−70	7 ± 1	94 ± 2
DMPP	1	14	−70	8 ± 1	95 ± 2
Nicotine	1	7	−70	9 ± 1	96 ± 2
Cytisine	1	11	−70	6 ± 1	96 ± 2

of the fast and slow currents varied from cell to cell, with some neurons exhibiting large (>300 pA) responses and others, in the same culture, exhibiting small (<100 pA) or no responses of either type.

Both the rapidly desensitizing and the slowly activating current were evoked in a dose-dependent fashion, requiring micromolar concentrations of ACh. The EC_{50} was 129 ± 28 μM for the fast current, whereas it was 65 ± 7 μM for the slow response (Fig. 2). The Hill coefficients for the fast and slow currents were 1.3 and 1.5, respectively, suggesting that more than one molecule of agonist is required to evoke either response.

Current-voltage (I-V) plots for both currents revealed strong inward rectification. Over the range of negative membrane potentials, both I-V curves were nearly linear. However, neither current showed a clear outward component at membrane potentials up to +80 mV, making it difficult to measure a true reversal potential for these currents (Fig. 3). In some cells, the fast current exhibited a small outward response at potentials greater than +10 mV, suggesting a reversal potential near 0 mV (Fig. 3A). However, the slow current showed small inward responses in some neurons even at +50 mV. The shape of the I-V curves was not altered by changes in the chloride reversal potential, exchange of internal cesium for sodium, or the inclusion or removal of magnesium from the intracellular or extracellular solution.

nAChRs mediate the ACh responses. The inwardly rectifying I-V relationships are similar to neuronal nicotinic responses in a number of other cells (18–21). Consistent with this observation, both the fast and slow ACh responses were recorded in the presence of 1 μM atropine, a muscarinic receptor antagonist. However, as reported for nicotinic responses in other preparations (22), concentrations of atropine of >10 μM diminished these currents in a reversible fashion. The addition of 1 μM atropine to the extracellular solution did not change the shape of the I-V curves.

Both the fast and slow ACh responses were inhibited by dTC and mecamylamine. dTC (100 μM) blocked rapidly desensitizing currents evoked by 1 mM ACh by 81 ± 2% (n = 4) and inhibited nondesensitizing currents by 56 ± 6% (n = 5) (Fig. 4). At 1 mM, dTC inhibited both currents completely (fast current, n = 3; slow current, n = 6). Mecamylamine (100 μM) inhibited the fast and slow responses produced by 1 mM ACh by 69 ± 14% (n = 3) and 67 ± 8% (n = 7), respectively. At 1 mM, mecamylamine inhibited fast currents by 89 ± 5% (n = 5) and slow currents completely (n = 2).

Responses similar to those produced by ACh were elicited by the nicotinic agonists nicotine, DMPP, and cytisine (Fig. 5, A, B, and C). Currents induced by these agonists also exhibited I-

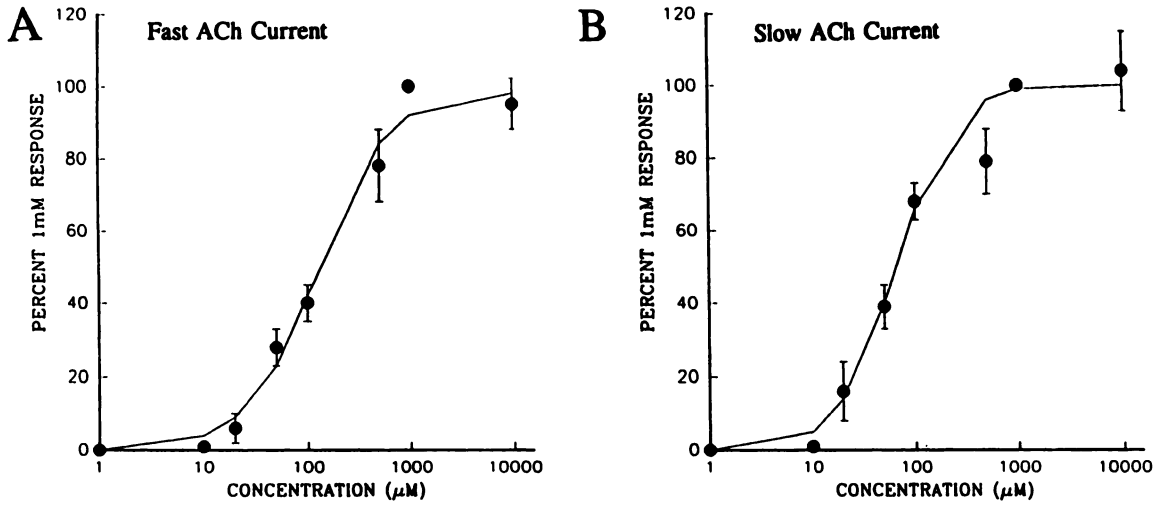

Fig. 2. ACh activates currents in a dose-dependent manner. Neurons exhibiting either the fast (rapidly desensitizing) current (A) or the slow current (B) were voltage-clamped at −70 mV and exposed to 100–500-msec applications of ACh. Each neuron was exposed to three ACh concentrations, and results were normalized with respect to the response at 1 mM. The *points* represent the mean ± standard error of three to seven cells/concentration. The *curves* represent the fit of the experimental data to a dose-response equation:

$$\text{Response} = \text{response}_{max} \times \frac{[ACH]^n}{[ACH]^n + EC_{50}{}^n}$$

using a least squares minimization routine. $Response_{max}$ is the maximal agonist response, EC_{50} is the half-maximal effective concentration, and n is the Hill coefficient. For the data in A, $response_{max}$ = 99 ± 6%, EC_{50} = 129 ± 28 μM, and n = 1.3 ± 0.2. For the data in B, $response_{max}$ = 100 ± 3%, EC_{50} = 65 ± 7 μM, and n = 1.5 ± 0.2.

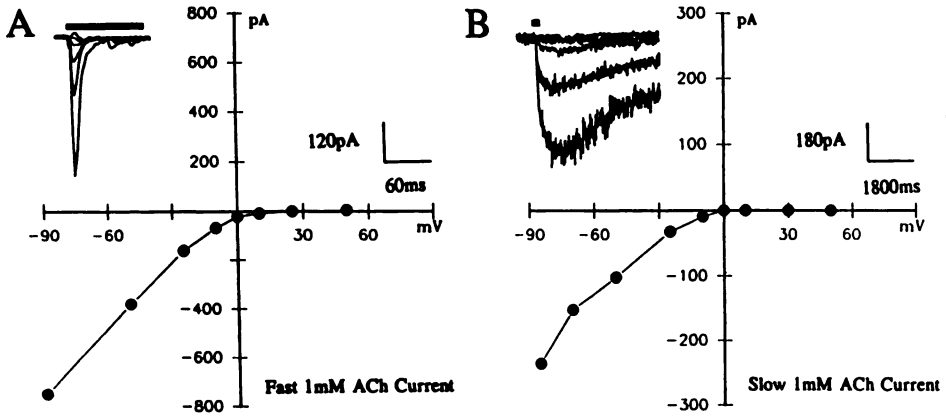

Fig. 3. ACh-gated currents have nonlinear I-V curves. Single neurons were voltage-clamped at various membrane potentials and exposed to 100-msec applications of 1 mM ACh. The graph shows the peak response at various membrane potentials over the range of −90 to +50 mV. *Insets*, raw data used in constructing the I-V curves. For clarity, the −85 and +50 traces are omitted from the *inset* in A, whereas the −70, +10, and +50 traces are omitted from the *inset* in B.

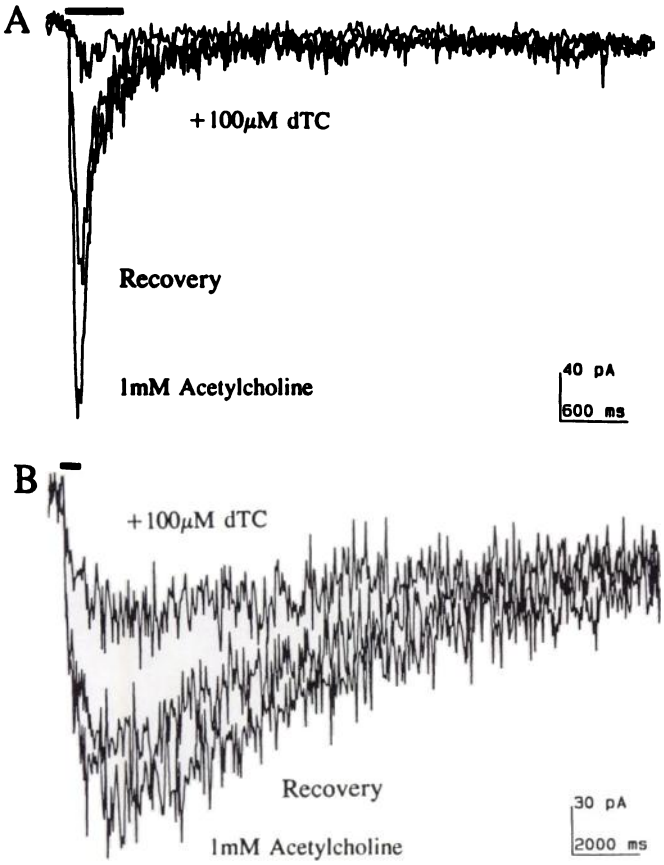

Fig. 4. dTC inhibits ACh currents. Two neurons were voltage-clamped at −70 mV and exposed to 500-msec applications of 1 mM ACh, in the absence and presence of 100 μM dTC. A, Response of a cell with a desensitizing current; B, response of a neuron exhibiting the slow ACh current. The data in this figure were obtained using pressure-ejection drug delivery.

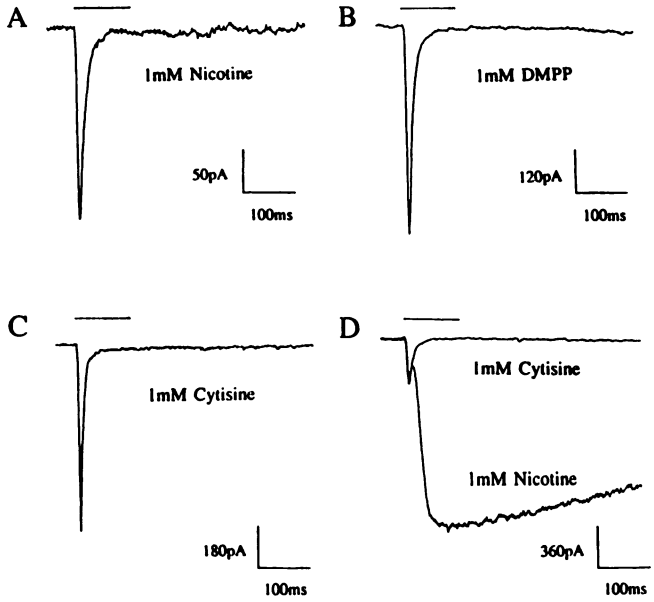

Fig. 5. Nicotine, DMPP, and cytisine gate rapidly desensitizing currents. The traces show the response of single neurons to 100-msec flow-tube applications of 1 mM nicotine (A), 1 mM DMPP (B), and 1 mM cytisine (C). D, In a single neuron, a 100-msec application of 1 mM nicotine and 1 mM cytisine evoked markedly different responses. The response to nicotine shows both the rapidly desensitizing and the slow response. In contrast, cytisine induced only a rapidly desensitizing current. Similar results were seen in two other cells. The holding potential for all traces in this figure was −70 mV.

V curves that, like ACh-gated responses, strongly rectified. The rate and degree of desensitization produced by 1 mM ACh, nicotine, DMPP, and cytisine were similar (Table 1). In 17 of 44 neurons studied, 1 mM nicotine elicited slow currents, whereas 8 of 49 cells exhibited slow currents in response to 1 mM DMPP. Interestingly, 1 mM cytisine induced either a rapidly desensitizing current ($n = 14$) or no response ($n = 17$) in 31 neurons. In 26 of these neurons, the effects of 1 mM nicotine and 1 mM cytisine were compared. Three of the 26 neurons exhibited both desensitizing and slow responses to 1 mM nicotine, whereas 1 mM cytisine evoked only rapidly desensitizing currents (Fig. 5D). Thus, cytisine may be a more selective agonist for the nAChRs mediating the fast current.

αBTXN inhibits rapidly desensitizing currents. In the hippocampus, the majority of presumed nAChRs bind αBTXN (1). We found that 1–2 μM αBTXN, applied by local perfusion, selectively blocked the rapidly desensitizing currents produced by 1 mM ACh, 1 mM DMPP, or 1 mM nicotine, while having little effect on slow currents (Fig. 6). After treatment with αBTXN, fast desensitizing currents were 8 ± 3% ($n = 16$) of control, whereas slow currents were 95 ± 3% ($n = 7$) of control. Consistent with binding studies indicating a slow off-rate for αBTXN binding (23), we found that the effects of the toxin were not reversible over a 0.5–1-hr period.

Because the irreversible block of currents makes it uncertain whether the decline in response is a pharmacological effect of αBTXN or the result of a nonspecific change in the response, we also examined whether a 15–30-min bath application of 1

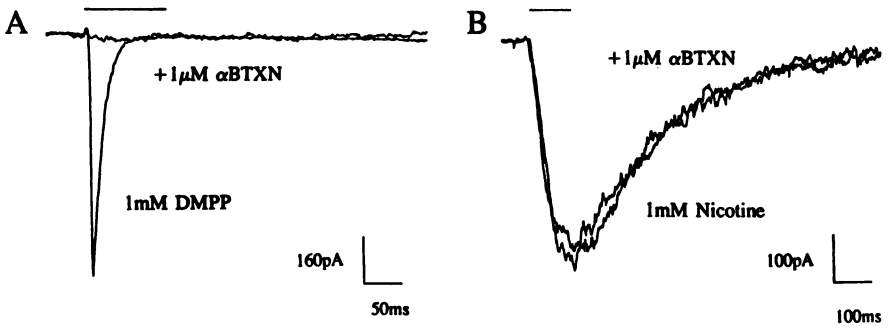

Fig. 6. αBTXN inhibits rapidly desensitizing nicotinic currents. A, The neuron was voltage-clamped at −70 mV, and 1 mM DMPP was applied by flow tube for 100 msec before and after exposure to 1 μM αBTXN for 60 sec by local perfusion. B, The neuron was voltage-clamped at −70 mV and exposed to 100-msec flow-tube applications of 1 mM nicotine before and after 1 μM αBTXN. In this cell, αBTXN was administered by bath perfusion for 15 min.

μM αBTXN altered the percentage of neurons exhibiting rapidly desensitizing responses. DMPP (1 mM) evoked rapidly desensitizing currents in 75% of neurons (15 of 20) from three separate cultures before exposure to the toxin. After αBTXN treatment, only 11% (2 of 18) displayed any desensitizing current in response to 1 mM DMPP, and the responses seen were of low amplitude, compared with control currents elicited by the same concentration administered before αBTXN. Before αBTXN fast desensitizing currents averaged 236 ± 26 pA (n = 15), whereas after αBTXN the average response of neurons showing a desensitizing response was 30 pA (n = 2).

Discussion

These results indicate that nicotinic agonists activate two types of current in cultured postnatal rat hippocampal neurons. The more prevalent response desensitized by >90%, with a time constant of <10 msec, in response to saturating ACh concentrations and was inhibited by αBTXN. The second class of responses activated more slowly and was insensitive to αBTXN. The reasons for the differences in apparent activation kinetics of the two currents are uncertain. It is possible that the activation reflects differences in receptor binding and/or channel opening rates. Additionally, differences in receptor localization on dendrites or soma could contribute. However, the finding of a slow onset of one class of response during flow-tube agonist applications, which achieve a rapid and fairly uniform solution exchange, suggests that receptor localization is not a major factor. Similarly, it is unlikely that the differences in kinetics are related to electrotonic coupling of neurons, given the differences in pharmacology and the fact that <3% of neurons in our cultures exhibit either electrical or dye coupling.[1] The fast and slow nicotinic responses also appear to differ with respect to their associated membrane noise. This may reflect differences in the primary single-channel conductances or channel open probabilities underlying the two responses (24).

The two currents are similar in a number of respects. As is true of neuronal nicotinic responses in other preparations, both have Hill coefficients of >1, suggesting that two agonist molecules are required to gate responses. Additionally, both responses are mediated by relatively low affinity receptors, which have EC_{50} values for ACh of >50 μM, and are sensitive to inhibition by dTC and mecamylamine. Our results suggest that the fast desensitizing current is more sensitive to block by 100 μM dTC, whereas both currents are equally sensitive to 100 μM mecamylamine. Previous studies have shown that nicotinic responses in different CNS regions have varying sensitivity to inhibition by these antagonists (10, 20, 25), consistent with the differences found in hippocampal neurons.

Both classes of nicotinic currents exhibit nonlinear I-V curves, with marked inward rectification. The channels mediating these currents are most likely cation selective, because changes in the chloride equilibrium potential had no effect on I-V curves. A true reversal potential could not be measured for either the fast or the slow current because of the failure to record clear outward currents at potentials up to +80 mV. The mechanisms underlying the nonlinear I-V relationships observed for hippocampal nicotinic responses are uncertain. In PC12 cells, blockade of nicotinic channels by intracellular magnesium contributes to the inward rectification (19). However, in hippocampal neurons, the presence or nominal absence of magnesium in intracellular or extracellular solutions had no effect on the shape of the I-V curves. In cultured midbrain neurons, nicotinic currents also inwardly rectify. The rectification in these cells is removed when responses are recorded in the presence of atropine, suggesting that a muscarinic receptor-mediated response masks the nicotinic component at positive membrane potentials (18). However, nicotinic channels in hippocampal neurons did not pass outward current in the presence of 1 μM atropine. Other possible reasons for the shape of the I-V curves include channel block by intracellular cesium ions (26) or other ions in solution. In our experiments, substituting sodium for cesium in the intracellular solution did not alter the inward rectification. Attempts to use potassium as the predominant intracellular cation were unsuccessful, due to large increases in base-line membrane noise at positive holding potentials. An alternative possibility is that voltage-dependent gating properties of the nicotinic ion channels may determine the shape of the I-V curves (19, 21).

Our results indicate that one class of hippocampal ACh responses desensitizes in <10 msec in the presence of saturating agonist concentrations. Rapidly desensitizing nicotinic responses have been described in several other preparations. In cultured midbrain neurons, ACh responses decay rapidly (18), although estimates of the rate of desensitization were not presented. Interestingly, a minority of midbrain neurons also exhibit slow, nondesensitizing, ACh responses, like the hippocampal neurons. Studies using rapid drug-application techniques have also found desensitization rates of <100 msec for nAChRs in bovine adrenal chromaffin cells (27), BC3H-1 cells (28), and adult mouse and frog interossal muscle cells (29).

Muscle nAChR channels are also subject to ion channel block by high concentrations of agonists. This channel block could contribute to the decay of responses at high agonist concentrations (30). Our data do not permit a determination of the relative contribution of desensitization and agonist-induced

[1] C. F. Zorumski and L. L. Thio, unpublished observations.

channel block to the decline in hippocampal nicotinic currents. Consistent with either desensitization or open channel block, both the rate and degree of desensitization increased with increasing ACh concentration. However, the lack of voltage dependence over the range of −90 to −20 mV would suggest that a simple open channel block mechanism cannot explain the results, because the ACh molecule is positively charged at physiological pH and might be expected to act at a site that is sensitive to the transmembrane electrical field.

An interesting feature of the fast desensitizing ACh current in hippocampal neurons is its sensitivity to αBTXN. α-Cobratoxin binding sites are present in the rodent hippocampus but have an unknown physiological function. Our data and those of Alkondon and Albuquerque (13) indicate that these receptors gate cationic currents. The apparent rapid activation and decay of these responses, similar to responses mediated by glutamate at ionotropic quisqualate receptors (17), would be consistent with a fast postsynaptic receptor. However, a fast nicotinic synaptic response has not been reported in hippocampal preparations.

Although the subunit composition of specific neuronal nAChRs is uncertain, several cloned receptor subunits, including αBTXN binding subunits, are expressed in the hippocampus (4–7). Among the neuronal nAChR subunits cloned to date, the rapidly desensitizing hippocampal current most closely resembles responses produced by expression of an αBTXN-binding protein, designated α7, isolated from chicken brain (31). This subunit encodes a protein sequence nearly identical to the protein sequence reported for an αBTXN-binding protein isolated from chick optic lobe (32). When expressed in *Xenopus* oocytes, the α7 subunit forms a homo-oligomeric ion channel that exhibits low affinity for ACh (EC_{50} = 115 μM), rapid and nearly complete desensitization, an inwardly rectifying I-V curve, and blockade by αBTXN. These properties are all seen with the fast hippocampal current. This αBTXN-binding protein is expressed in rat brain, and preliminary results suggest a distribution similar to that of ^{125}I-αBTXN binding (7). Our results suggest that αBTXN-sensitive receptors form ion channels in vertebrate neurons but that these channels are subject to rapid desensitization, making them difficult to study in experiments using slower drug-delivery methods.

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References

1. Clarke, P. B. S., R. D. Schwartz, S. M. Paul, C. B. Pert, and A. Pert. Nicotinic binding in rat brain: autoradiographic comparison of [^{3}H]acetylcholine, [^{3}H]nicotine, and [^{125}I]-α-bungarotoxin. *J. Neurosci.* **5**:1307–1315 (1985).
2. Brown, D. A., and L. Fumagalli. Dissociation of α-bungarotoxin binding and receptor block in the rat superior cervical ganglion. *Brain Res.* **129**:165–168 (1977).
3. Luetje, C. W., J. Patrick, and P. Seguela. Nicotinic receptors in the mammalian brain. *FASEB J.* **4**:2753–2760 (1990).
4. Deneris, E. S., J. Connolly, J. Boulter, E. Wada, K. Wada, L. W. Swanson, J. Patrick, and S. Heinemann. Primary structure and expression of β2: a novel subunit of neuronal nicotinic acetylcholine receptors. *Neuron* **1**:45–54 (1988).
5. Goldman, D., E. Deneris, W. Luyten, A. Kochhar, J. Patrick, and S. Heinemann. Members of a nicotinic acetylcholine receptor gene family are expressed in different regions of the mammalian central nervous system. *Cell* **48**:965–973 (1987).
6. Wada, E., K. Wada, J. Boulter, E. Deneris, S. Heinemann, J. Patrick, and L. W. Swanson. Distribution of *alpha2*, *alpha3*, *alpha4*, and *beta2* neuronal nicotinic receptor subunit mRNAs in the central nervous system: a hybridization histochemical study in the rat. *J. Comp. Neurol.* **284**:314–335 (1988).
7. Schoepfer, R., W. G. Conroy, P. Whiting, M. Gore, and J. Lindstrom. Brain α-bungarotoxin binding protein cDNAs and MAbs reveal subtypes of this branch of the ligand-gated ion channel gene superfamily. *Neuron* **5**:35–48 (1990).
8. Ropert, N., and K. Krnjevic. Pharmacological characteristics of facilitation of hippocampal population spikes by cholinomimetics. *Neuroscience* **8**:1963–1977 (1982).
9. Rovira, C., Y. Ben-Ari, E. Cherubini, K. Krnjevic, and N. Ropert. Pharmacology of the dendritic action of acetylcholine and further observations on the somatic disinhibition in the rat hippocampus *in situ*. *Neuroscience* **8**:97–106 (1983).
10. Freund, R. K., D. A. Jungschaffer, and A. C. Collins. Nicotinic effects in mouse hippocampus are blocked by mecamylamine but not other nicotinic antagonists. *Brain Res.* **511**:187–191 (1990).
11. Miner, L. L., M. J. Marks, and A. C. Collins. Relationship between nicotine-induced seizures and hippocampal nicotinic receptors. *Life Sci.* **37**:75–83 (1985).
12. Arcava, Y., S. S. Deshpande, K. L. Swanson, H. Rapoport, S. Wonnacott, G. Lunt, and E. X. Albuquerque. Nicotinic acetylcholine receptors in cultured neurons from the hippocampus and brain stem of the rat characterized by single channel recording. *FEBS Lett.* **222**:63–70 (1987).
13. Alkondon, M., and E. X. Albuquerque. α-Cobratoxin blocks the nicotinic acetylcholine receptor in rat hippocampal neurons. *Eur. J. Pharmacol.* **191**:505–506 (1990).
14. Huettner, J. E., and R. W. Baughman. Primary culture of identified neurons from the visual cortex of postnatal rats. *J. Neurosci.* **6**:3044–3060 (1986).
15. Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. Improved patch-clamp techniques for high resolution current recordings from cells and cell-free membrane patches. *Pflügers Arch.* **391**:85–100 (1981).
16. Mayer, M. L., and L. Vyklicky. Concanavalin A selectively reduces desensitization of mammalian neuronal quisqualate receptors. *Proc. Natl. Acad. Sci. USA* **86**:1411–1415 (1989).
17. Trussell, L. O., L. L. Thio, C. F. Zorumski, and G. D. Fischbach. Rapid desensitization of glutamate receptors in vertebrate central neurons. *Proc. Natl. Acad. Sci. USA* **85**:2834–2838 (1988).
18. Bijak, M., W. Jarolimek, and U. Misgeld. Effects of antagonists on quisqualate and nicotinic-mediated currents of midbrain neurones in culture. *Br. J. Pharmacol.* **102**:699–705 (1991).
19. Ifune, C. K., and J. H. Steinbach. Rectification of acetylcholine-elicited currents in PC12 pheochromocytoma cells. *Proc. Natl. Acad. Sci. USA* **87**:4794–4798 (1990).
20. Mulle, C., and J. P. Changeux. A novel type of nicotinic receptor in the rat central nervous system characterized by patch-clamp techniques. *J. Neurosci.* **10**:169–175 (1990).
21. Mathie, A., D. Colquhoun, and S. G. Cull-Candy. Rectification of currents activated by nicotinic acetylcholine receptors in rat sympathetic ganglion neurones. *J. Physiol.* (Lond.) **427**:625–655 (1990).
22. Sadoshima, J., Y. Oyama, and N. Akaike. Inhibition of nicotinic acetylcholine responses by atropine in frog isolated sympathetic neurons. *Brain Res.* **508**:147–151 (1990).
23. Lukas, R. J. Properties of curare-mimetic neurotoxin binding sites in the rat central nervous system. *Biochemistry* **23**:1152–1160 (1984).
24. Sigworth, F. J. The variance of sodium current fluctuations at the node of Ranvier. *J. Physiol.* (Lond.) **307**:97–129 (1980).
25. Mulle, C., C. Vidal, P. Benoit, and J.-P. Changeux. Existence of different subtypes of nicotinic acetylcholine receptors in the rat habenulo-interpeduncular system. *J. Neurosci.* **11**:2588–2597 (1991).
26. Hirano, T., Y. Kidokoro, and H. Ohmori. Acetylcholine dose-response relations and the effect of cesium ions in the rat adrenal chromaffin cell under voltage clamp. *Pflügers Arch.* **408**:401–407 (1987).
27. Maconochie, D. J., and D. E. Knight. A method for making solution changes in the sub-millisecond range at the tip of a patch pipette. *Pflügers Arch.* **414**:589–596 (1989).
28. Dilger, J. P., and R. S. Brett. Direct measurement of the concentration- and time-dependent open probability of the nicotinic acetylcholine receptor channel. *Biophys. J.* **57**:723–731 (1990).
29. Franke, C., H. Hatt, and J. Dudel. Steep concentration dependence and fast desensitization of nicotinic channel currents elicited by acetylcholine pulses, studied in adult vertebrate muscle. *Pflügers Arch.* **417**:509–516 (1991).
30. Sine, S. M., and J. H. Steinbach. Agonists block currents through acetylcholine receptor channels. *Biophys. J.* **46**:277–284 (1984).
31. Couturier, S., D. Bertrand, J.-M. Matter, M.-C. Hernandez, S. Bertrand, N. Millar, S. Valera, T. Barkas, and M. Ballivet. A neuronal nicotinic acetylcholine receptor subunit (α7) is developmentally regulated and forms a homo-oligomeric channel blocked by α-BTX. *Neuron* **5**:847–856 (1990).
32. Conti-Tronconi, B. M., S. M. J. Dunn, E. A. Barnard, J. O. Dolly, F. A. Lai, N. Ray, and M. A. Raftery. Brain and muscle nicotinic acetylcholine receptors are different but homologous proteins. *Proc. Natl. Acad. Sci. USA* **82**:5208–5212 (1985).

Send reprint requests to: Charles F. Zorumski, Department of Psychiatry, Washington University School of Medicine, 4940 Audubon Avenue, St. Louis, MO 63110.