Pflügers Arch (1987) 410:37–43

Pflügers Archiv
European Journal of Physiology


# Neural nicotinic acetylcholine responses in solitary mammalian retinal ganglion cells

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**Abstract.** Using the patch-clamp technique, whole-cell recordings from solitary rat retinal ganglion cells in culture have established the nicotinic nature of the acetylcholine responses in these central neurons. Currents produced by acetylcholine (5–20 μmol/l) or nicotine (5–20 μmol/l) reversed in polarity near −5 mV and were unaffected by atropine (10 μmol/l). Agonist-induced currents were blocked by low doses (2–10 μmol/l) of the classical 'ganglionic' antagonists hexamethonium and mecamylamine, as well as by d-tubocurarine and dihydro-β-erythroidine (the latter two do not discriminate clearly between ganglionic and neuromuscular junction receptors). Treatment with the potent neuromuscular blocking agent α-bungarotoxin (10 μmol/l) did not affect the cholinergic responses of these cells, while toxin F (0.2 μmol/l), a neural nicotinic receptor antagonist, readily abolished acetylcholine-induced currents. Thus, the experiments performed to date show that the nicotinic responses of retinal ganglion cells in the central nervous system share the pharmacology of autonomic ganglion cells in the peripheral nervous system. The ionic current carried by the nicotinic channels was selective for cations, similar to that described for nicotinic channels in other tissues. In addition, single-channel currents elicited by acetylcholine were observed in whole-cell recordings with seals > 5 GΩ as well as in occasional outside-out patches of membrane. These acetylcholine-activated events, which had a unitary conductance of 48 pS and a reversal potential of 0 mV, represent the ion channels that mediate the neural nicotinic responses observed in these experiments on retinal ganglion cells.

**Key words:** Patch clamp – Rat central nervous system – Toxin F – α-Bungarotoxin – d-Tubocurarine – Mecamylamine – Hexamethonium – Dihydro-β-erythroidine

## Introduction

Nicotinic acetylcholine receptors are thought to exist in the mammalian central nervous system based upon results of ligand binding studies and indirect physiological assays (reviewed in Morley and Kemp 1981; Neal 1983; Puro 1985). However, studies on isolated neurons from the mammalian central nervous system have not previously shown physiological responses to pharmacological agents specific for the nicotinic receptor. Recently, molecular biological evidence in rat brain based upon homologous RNA species to cDNA clones has further suggested the existence of a neural type of these receptors which is distinct from the receptors at the neuromuscular junction (Boulter et al. 1986). In the present report we describe whole-cell patch clamp experiments on solitary rat retinal ganglion cells in culture that demonstrate pharmacologically the presence of nicotinic electrical responses in these central neurons. These results are the first to show directly on isolated cells the properties of a nicotinic receptor from a mammalian central neuron. In addition, we have been able to record the single-channel events that underlie the responses mediated by this receptor. An abstract describing part of this work has appeared previously (Lipton et al. 1986).

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## Methods

Membrane current recordings were made using the patch-clamp technique from enzymatically isolated, fluorescently labelled retinal ganglion cells obtained from 7–12 day postnatal rat retinas as described previously (Leifer et al. 1984; Lipton 1986; Lipton and Tauck 1987). Briefly, a fluorescent dye (granular blue) was injected into the superior colliculus for retrograde transport to the retinal ganglion cells over a two day period. Then the retinas were dissociated and kept in short-term cultures of 1–3 days duration at 37° C. For electrophysiological recordings, the external bathing solution was changed from minimal essential medium supplemented with 5% rat or 7.5% (v/v) fetal calf serum to one with Hanks' salts containing (in mmol/l) NaCl 137; $NaHCO_3$ 1, $Na_2HPO_4$ 0.34, KCl 5.4, $KH_2PO_4$ 0.44, $CaCl_2$ 2.5, $MgSO_4$ 0.5, $MgCl_2$ 0.5, HEPES-NaOH 5, glucose 22.2, phenol red indicator (0.001%, v/v). A heated dish containing the cells was continuously superfused at 0.8 ml/min with this bath solution during the recording session. The dish contained a stainless steel insert which limited fluid volume in the dish to approximately 100 μl. Drug additions made to the superfusate resulted in maximal concentration change within approximately 1 min (± 15% from dye studies). More acute additions were accomplished by pressure ejection from micropipettes (tip diameter 5–10 μm) placed 10–30 μm from the cell. Only solitary ganglion cells (identified by their fluorescence) were used in the present study ($n$ = 214); they were nearly spherical, lacked processes, and varied in diameter from 5–20 μm. Membrane currents were recorded in the 'whole-cell' and 'outside-out'

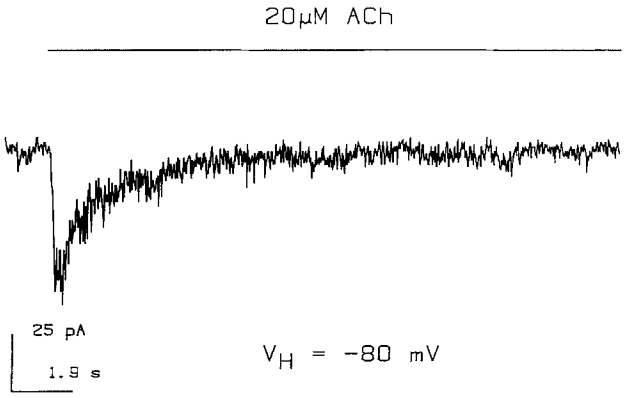


**Fig. 1.** Whole-cell current response of a solitary retinal ganglion cell to acetylcholine (ACh). Under voltage clamp, inward membrane current was induced by acetylcholine applied by pressure ejection from a micropipette when the holding potential ($V_H$) was −80 mV. This cell had an input resistance of 1.1 GΩ and cell capacitance of 4.5 pF (calculated by using small hyperpolarizing pulses). This round cell's diameter was 12 μm, yielding a specific membrane capacitance of approximately $10^{-6}$ F cm$^{-2}$. The series resistance was 11 MΩ. The ganglion cell was bathed in a normal physiological saline solution (see Methods) which contained 10 μmol/l atropine. The pipette solution was composed of a KCl-based saline (see Methods). The *bar* above the current trace represents the period of drug addition by pressure ejection in this and in subsequent figures

patch modes (Hamill et al. 1981) at 32–35° C. A detailed account of the recording procedures can be found in Lipton and Tauck (1987). Briefly, the currents measured with an EPC-7 amplifier (List Electronic, Darmstadt, FRG) were digitized at a rate of 0.1–1.2 ms per point by a PDP-11/23 or 11/73 computer (Digital Equipment Corporation) with Hewlett-Packard display. Prior to digitization the signals were filtered at a frequency of 0.5–2 kHz, appropriate to the rate of data acquisition. Data from records lasting many seconds in duration were commonly collected directly into computer memory and redisplayed using only every sixteenth point for ease of measurement. The additional points were useful for high temporal resolution of single channels. The intracellular (pipette) solution usually contained (in mmol/l) KCl 140, $MgCl_2$ 1, HEPES-NaOH 10, EGTA 1.5, $CaCl_2$ 1 (calculated free [$Ca^{2+}$] was 200 nmol/l). In some instances equimolar CsCl or 120 mmol/l CsCl plus 20 mmol/l tetraethylammonium chloride (TEACl) was substituted for KCl. All solutions were buffered to pH 7.2.

## Results

### *Effects of cholinergic drugs on whole-cell currents*

Figure 1 shows that when the bath and recording pipette contained solutions with physiological concentrations of cations, acetylcholine (5–20 μmol/l) evoked an inward, rapidly desensitizing current with the cell voltage-clamped at the resting potential (−60 to −80 mV; Leifer et al. 1984; Lipton and Tauck 1987). The whole-cell current was unaltered by 10 μmol/l atropine which was continuously present in the superfusion medium. The rate of desensitization varied from cell to cell, but lower concentrations of agonist produced less desensitization in any given cell. Acetylcholine-induced currents remained inward at holding potentials between −80 and −5 mV (Figs. 1, 2).

The addition of 2–10 μmol/l d-tubocurarine reversibly blocked the effect of acetylcholine (Fig. 2A). In fact, d-tubocurarine was capable of completely antagonizing the current induced by acetylcholine even in the absence of atropine, suggesting that the entire cholinergic effect was nicotinic under these conditions. The level of noise in the records sometimes increased during and for several minutes following the addition of d-tubocurarine. This phenomenon might reflect channel flicker from a partial agonist effect (Asher et al. 1979; Trautman 1982). In addition, nicotine (5–20 μmol/l; Fig. 2B) but not muscarine (40 μmol/l, not illustrated) mimicked the effect of acetylcholine. Figure 2B shows that the current generated by nicotine was rapidly and reversibly abolished by hexamethonium (10–20 μmol/l).

Figure 3 summarizes the effect of several pharmacological agents on the current-voltage relation of the peak whole-cell currents induced by acetylcholine. Atropine (10 μmol/l) had no effect on the cholinergic currents at any holding potential. In contrast, d-tubocurarine (10 μmol/l) and dihydro-β-erythroidine (10 μmol/l) suppressed the response to acetylcholine at all holding potentials. Among the classical ganglionic blocking agents, both hexamethonium (10 μmol/l) and mecamylamine (10 μmol/l) antagonized the effect of acetylcholine. Even at relatively low concentrations, these agents completely blocked cholinergic currents at each holding potential tested. We have not yet tried sufficiently low doses to test if the block is voltage dependent as in rat parasympathetic ganglion cells (Ascher et al. 1979).

It is noteworthy that in other experiments using culture dishes plated at high density and containing a specific batch of rat serum, the application of acetylcholine to retinal ganglion cells clamped at the resting potential (−60 mV) resulted in what appeared to be an outward rather than an inward current; nevertheless, this current had the same reversal potential as the current described above. Using a tritiated choline precursor, analysis of the incubation medium in some of these culture dishes revealed that low levels of acetylcholine were being released into the fluid (Frosch et al. 1986), producing a tonic activation of nicotinic channels. Under these conditions the addition of exogenous acetylcholine from the pressure ejection pipette led to inactivation of this tonic cholinergic inward current because of receptor desensitization (S. A. Lipton, unpublished observations). Thus, the apparent outward current induced by acetylcholine actually represented a decrease in an underlying inward current. These effects of endogenous acetylcholine in the culture dish will be considered elsewhere.

During the course of the present study we also sought possible muscarinic actions of acetylcholine (as described in other mammalian central neurons by Krnjevic et al. 1971; Halliwell and Adams 1982; Cole and Nicoll 1983; Brown 1986; Egan and North 1986b; McCormick and Prince 1986). We did not encounter effects of muscarine (40 μmol/l) during whole-cell recording of retinal ganglion cells bathed in normal medium or in 30 mmol/l $[K^+]_0$ even when GTP (100 μmol/l), ATP (1.5 mmol/l); cyclic AMP (100 μmol/l), and cyclic GMP (100 μmol/l) were added to a pipette solution containing K-saline with or without $Mg^{2+}$ (data not illustrated). These agents were intended to reverse possible 'washout' of intracellular components critical for certain currents, such as M-current (Galvan et al. 1984; Breitwieser and Szabo 1985; Pfaffinger et al. 1985). Under the conditions of the present experiments, these findings, coupled with the absence of a blocking effect of atropine, indicate that the

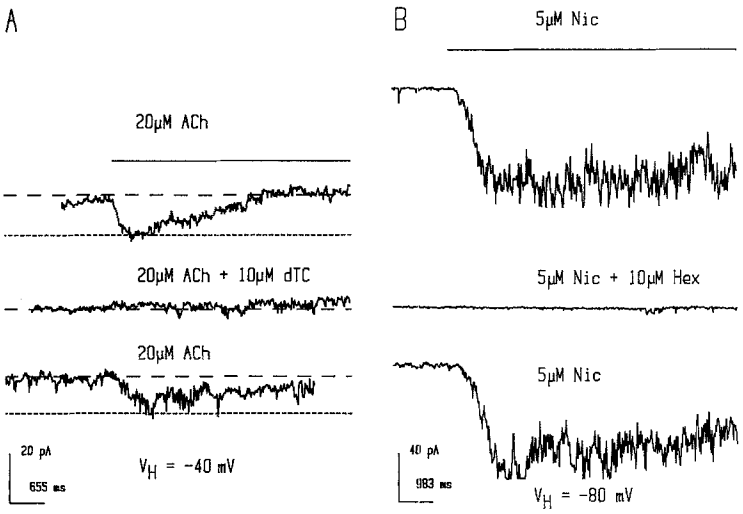


**Fig. 2A, B**
Whole-cell currents in retinal ganglion cells in response to acetylcholine (*ACh*) or nicotine (*Nic*) are blocked by d-tubocurarine (*dTC*) or hexamethonium (*Hex*). **A** Responses of a cell held at −40 mV to acetylcholine applied by pressure ejection from one micropipette before and after co-application of d-tubocurarine with acetylcholine from a second micropipette containing a mixture of the two drugs. The initial portion of the top trace appears slightly below the baseline because the current was still recovering from the previous application of acetylcholine. CsCl plus TEACl were substituted for KCl in the pipette-filling solution (see Methods). **B** Responses of another cell held at −80 mV to nicotine applied by one micropipette before and after co-application of hexamethonium and nicotine from a second micropipette. In the lowest trace, the peak current was slightly clipped because the signal exceeded the range of the analog-to-digital converter at this gain. The pipette contained the KCl solution

responses of retinal ganglion cells to acetylcholine were nicotinic and not muscarinic.

*Effects of toxins on nicotinic currents*

In 10 experiments, α-bungarotoxin (10 nmol/l to 10 μmol/l; purified by a procedure described by Lee et al. 1972) was added to the bath solution superfusing the ganglion cells for up to 1 h. The toxin, which eliminates acetylcholine-induced current at the neuromuscular junction but not in autonomic ganglia (Brown and Fumagalli 1977), had no effect on these responses in mammalian retinal ganglion cells that were stable for long periods of time (Fig. 4A). At first, this finding appeared at odds with an earlier report that the venom from *Bungarus multicinctus* rapidly abolished the light responses of ganglion cells recorded in the isolated rabbit retina with extracellular electrodes (Masland and Ames 1976). However, recently it has been determined that the crude snake venom consists of several toxins and that one of them, toxin F (Loring et al. 1984), which is identical to bungarotoxin 3.1 (Ravdin and Berg 1979) and κ-bungarotoxin (Chiappinelli 1983), blocks the neural nicotinic responses of autonomic ganglion cells (Sah et al. 1987). Therefore, it is possible that this component of the venom was responsible for the nicotinic blockade in the mammalian retina. We tested this hypothesis by superfusing solitary retinal ganglion cells in toxin F (0.2 μmol/l; purified as described by Loring et al. 1986) and found that it was a potent antagonist of acetylcholine in retinal ganglion cells (Fig. 4B). The effect of toxin F was only partly reversible, with recovery resulting in single-channel currents during whole-cell recordings (bottom trace of Fig. 4B, and Fig. 5). Washing for over 1 h did not appear to increase the degree of recovery.

*Ionic selectivity of central nicotinic channels*

Analysis of the ionic selectivity of the cholinergic current in rat retinal ganglion cells was complicated by the fact that it was fairly small in amplitude (range 6–100 pA at a holding potential of −80 mV). If the $K^+$ currents were not adequately suppressed during depolarization, the large $K^+$ currents in these cells (Lipton and Tauck 1987) would mask the smaller responses to acetylcholine. To alleviate this problem, current-voltage relationships were obtained using $Cs^+$ (often with TEA) as the major cation in the pipette (see legend to Fig. 2A). Under these conditions, the reversal potential of the cholinergic current was $-5.3 \pm 2.4$ mV (mean ± SD, $n = 12$). This finding is consistent with ability to a nonselective cation or anion current. Substitution for $Cl^-$ in the pipette with acetate, aspartate or 2[N-morpholino]ethanesulfonate did not effect the reversal potential (not illustrated, $n = 6$). These results suggest that the current induced by acetylcholine is relatively nonselective for cations, and is similar to that observed for nicotinic channels in embryonic muscle (Hamill and Sakmann 1981) and in adrenal chromaffin cells (Fenwick et al. 1982).

*Single-channel recordings of nicotinic current*

Not all retinal ganglion cells displayed comparable cholinoreceptive properties. Although the majority of cells responded to acetylcholine, the magnitude of the response varied widely, perhaps reflecting the variable sensitivity of different types of ganglion cells in vivo (Ariel and Daw 1982a, b; Ikeda and Sheardown 1982). During whole-cell recording with high resistance seals (> 5 GΩ) from some cells with apparently only a few active cholinergic receptors on their surface, single channels could be discerned when a nicotinic cholinergic agonist was applied. Also, single-channel events were evident when larger whole-cell current responses to acetylcholine had desensitized substantially so that only a single channel was open at a time. However, the most reliable period to observe single cholinergic channels occurred during the recovery phase after exposure to toxin F. Thus, toxin F, analogous to α-bungarotoxin in muscle, could be used to reduce the number of active channels and make unitary events more discernible. For example, Fig. 5A shows the response to 50 μmol/l acetylcholine during a whole-cell recording at different holding potentials. During comparable intervals both before and 2 min after the exposure to acetylcholine, there were no channel openings. The total amplitude histogram of channel activity in the presence of acetylcholine over a period of approximately 18 s at a holding potential of −60 mV is illustrated in Fig. 5B. The peaks of the histograms obtained at each holding potential were used to determine the mean of the single-channel current amplitude at that voltage. Using these figures, the current-voltage relation for a single acetyl-

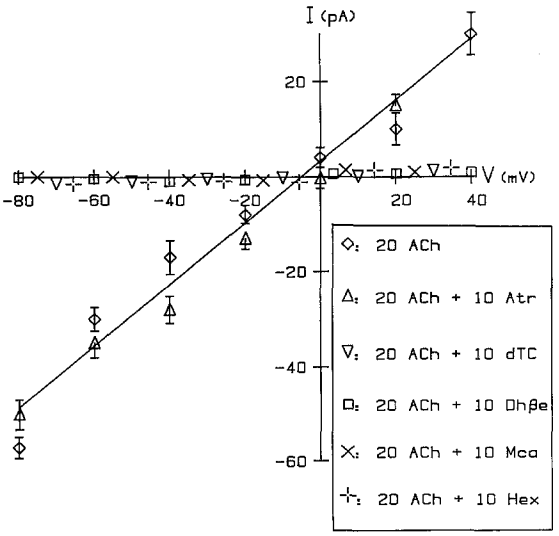


**Fig. 3.** Current-voltage relation of peak whole-cell currents from a retinal ganglion cell responding to cholinergic drugs (same cell as in Fig. 2A). The peak currents were measured by computer as shown in Fig. 2A using the dashed baseline and dotted peak-current line. Concentrations given are in μmol/l. The data points are the means and the bars, the standard deviation based upon 5 observations. The least-squares fit curve applies to the responses to acetylcholine alone. The curve has a slope conductance of 0.65 nS and a reversal potential $E_r = -5.0$ mV. For the various blocking drugs, the data points near 0 pA have been displaced laterally for clarity. Usually 1–2 min were allowed for recovery between applications of drugs. To test for reversibility, the order of addition of the test agents was acetylcholine (*ACh*), acetylcholine + d-tubocurarine (*dTC*), acetylcholine, acetylcholine + dihydro-β-erythroidine (*Dhβe*), acetylcholine, acetylcholine + mecamylamine (*Mca*), acetylcholine, acetylcholine + hexamethonium (*Hex*), acetylcholine, acetylcholine + atropine (*Atr*). Acetylcholine, acetylcholine + d-tubocurarine, and acetylcholine + atropine were applied with three separate pressure-ejection pipettes. The other blockers were added to the superfusate bathing the cell in this experiment but were applied by pneumatic pipettes to other cells, yielding similar results. An accurate measurement of the peak current was critical to avoid an artifactual sublinear I–V curve. Accordingly, the rise time of the acetylcholine-induced transient had to be significantly faster than the decay time of the response. This condition was met in all the cells that we analyzed

choline-activated channel was produced (Fig. 5C). The reversal potential was near 0 mV and the single-channel conductance, obtained from the slope of the fitted line, was 48 pS at 35° C. Similar conductances were obtained in 7 other cells (48.9 ± 3.5 pS, mean ± SD). The unitary conductance measured in whole-cell recordings displaying discrete single channel events in the absence of toxin F was of similar amplitude ($n = 3$). Single nicotinic channels were also observed in outside-out patches from retinal ganglion cells, but their occurrence was relatively rare. Five of 67 (7.5%) of these patches contained channels that were activated by low concentrations of acetylcholine (200–300 nmol/l). Inclusion of atropine (10 μmol/l) in the bathing medium had no noticeable effect on the acetylcholine-induced channels ($n = 4$).

## Discussion

The acetylcholine-induced current observed in the present study represents the first direct assay of a functional nicotinic receptor in an identified neuron isolated from the mammalian central nervous system. Previous work in mammals had established the presence of central nicotinic receptors by both extracellular and intracellular recording techniques in retina (Masland and Ames 1976; Ariel and Daw 1982a, b; Ikeda and Sheardown 1982), brain (Brown et al. 1983; Hatton et al. 1983; Clark et al. 1985; Egan and North 1986a), and spinal cord (Eccles et al. 1954; Duggan et al. 1976). We find the characteristics of the electrical responses of rat retinal ganglion cells that are mediated by this receptor are the following: (1) a pharmacology similar to the nicotinic responses of autonomic ganglion cells in the peripheral nervous system but different from that of the neuromuscular junction; (2) a lack of sensitivity to block by α-bungarotoxin; (3) a potent antagonism by toxin F; and (4) an associated ionic channel that is relatively nonselective for cations and has a unitary conductance of 48 pS at 35° C.

For the most part muscarinic rather than nicotinic effects have been reported in the mammalian central nervous system (reviewed by Brown 1979). The actions of muscarine include: inhibiting a slow $Ca^{2+}$-activated $K^+$ conductance (Cole and Nicoll 1983), switching off a slow voltage-dependent $K^+$ current (M-current; Krnjevic et al. 1971; Halliwell and Adams 1982), activating a cation conductance (cited in Brown 1986), or enhancing a $K^+$ conductance (McCormick and Prince 1986; Egan and North 1986b). In the present study, however, no evidence for a muscarinic action was observed during whole-cell or excised patch recordings of rat retinal ganglion cells. Atropine had no effect on the currents activated by acetylcholine. Nevertheless, our experimental conditions do not preclude a muscarinic effect on retinal ganglion cells if a specific second messenger unknown to us and requisite for the action of acetylcholine on a muscarinic receptor had been dialyzed out of the cell by the contents of the patch electrode.

In contrast, we observed that the acetylcholine-induced currents in retinal ganglion cells were blocked by relatively low doses (2–10 μmol/l) of all of the nicotinic antagonists that were tested. This result held true regardless of the holding potential at which the cell was voltage clamped. These findings differ from those of Ascher et al. (1979) in the peripheral nervous system; in that study rat parasympathetic ganglion cells exhibited an increase in the degree of block by some antagonists (hexamethonium and d-tubocurarine) as the cell was hyperpolarized, suggestive of channel blockade. Whether or not these effects will become apparent in retinal ganglion cells at different antagonist concentrations will be the subject of future work. Nevertheless, the selective blockade of acetylcholine-activated currents in our preparation by classical ganglionic antagonists such as hexamethonium and mecamylamine suggests that the central nicotinic receptor studied here is pharmacologically similar to the peripheral ganglionic type. In addition, we found no evidence for blockade by α-bungarotoxin, an agent which strongly antagonizes nicotinic currents in muscle but not in mammalian neurons (reviewed by Brown 1979; Oswald and Freeman 1981). The absence of block by α-bungarotoxin is significant since this ligand has been used extensively as a marker for putative nicotinic receptors in the vertebrate retina (reviewed in Neal 1983; Puro 1985), as well as in other areas of the central nervous system (Morley and Kemp 1981). It should be noted however, that rat retina is relatively deficient in α-bungarotoxin binding sites (Shechter et al. 1978). Thus, the function of this binding site in the retinas

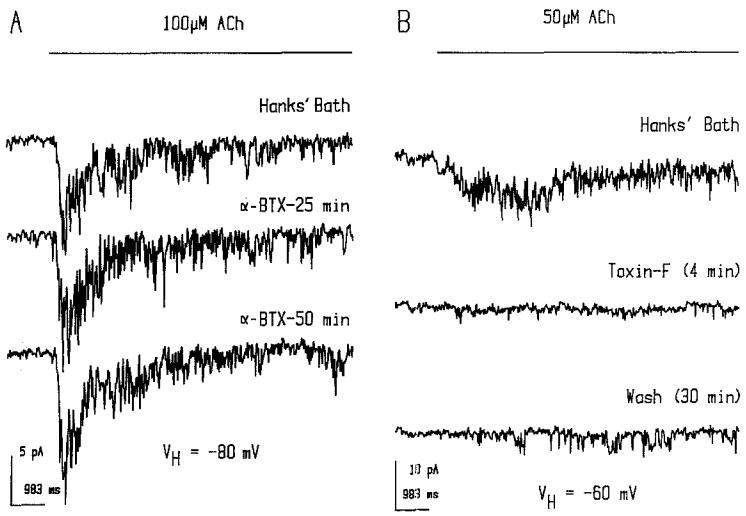


**Fig. 4A, B**
Responses to acetylcholine are blocked by toxin F but not by α-bungarotoxin during whole-cell recording. **A** Response recorded from a retinal ganglion cell after addition of acetylcholine *(ACh)* by a pressure-ejection pipette before and after α-bungarotoxin (*α-BTX*, 10 μmol/l) was introduced into the bath by superfusion for the period of time indicated. No effect on the ACh-induced current was observed. **B** In another retinal ganglion cell, superfusion with toxin F (0.2 μmol/l) for 4 min resulted in the obliteration of the response to ACh. Following a 30 min wash, ACh elicited single-channel currents signifying a partial recovery (see also Fig. 5). For both **A** and **B** the pipette solutions contained CsCl instead of KCl

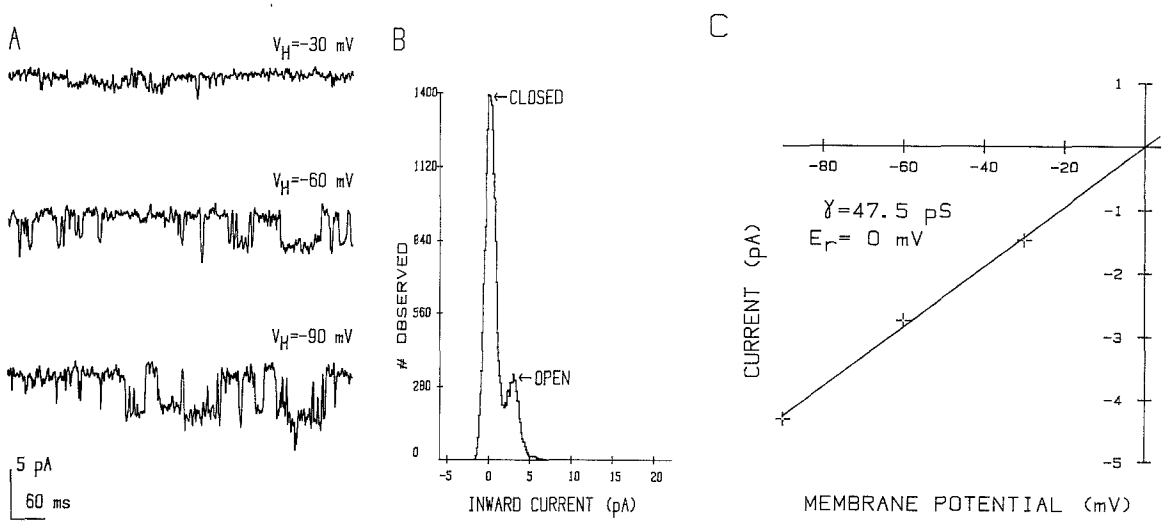


**Fig. 5A–C.** Single-channel currents induced by acetylcholine during whole-cell recording from a retinal ganglion cell. Toxin F (0.2 μmol/l) was used to reduce the number of active channels. **A** Responses to acetylcholine (50 μmol/l) applied by pressure ejection from a micropipette during the recovery phase after toxin F blockade. Openings of the acetylcholine-activated channels are shown as downward (inward) deflections of current. Channel openings were not seen in the absence of acetylcholine (not shown). **B** Histogram of current levels for the same cell as in **A** in the presence of acetylcholine (50 μmol/l) for about 10k points (sampled at 1.6 kHz and filtered at 500 Hz; membrane potential was held at −60 mV; the current level corresponding to the closed state was verified in records obtained in the absence of acetylcholine). The histogram shows the frequency of occurrence of the closed state (peak at 0 pA) as well as channel openings (peak at 2.8 pA). **C** Single-channel I–V plot for acetylcholine-induced events in the same cell as in **A**. The least-squares curve has a reversal potential $E_r$ = 0 mV and a slope conductance $\gamma$ = 47.5 pS. The coefficient of correlation for the fit was $r^2$ = 0.996. CsCl was substituted for KCl in the pipette solution

of other species remains to be determined. Our results prove the lack of a direct physiological effect of α-bungarotoxin on at least one type of mammalian central nicotinic receptor.

The inability of α-bungarotoxin to block nicotinic function at most autonomic ganglia (e.g., Brown and Fumagalli 1977) suggests that this toxin's binding site may not represent a functional neural nicotinic receptor. In contrast, toxin F (which is identical to bungarotoxin 3.1 and κ-bungarotoxin, Loring et al. 1986) does block cholinergic responses in the peripheral nervous system at a variety of autonomic ganglia (Loring et al. 1984; Chiappinelli and Dryer 1984; Sah et al. 1987). In addition, the binding of radiolabelled toxin F has been localized to nicotinic synapses in chick ciliary ganglia (Loring and Zigmond 1987). In the present study we found that toxin F was also effective in abolishing nicotinic currents in a mammalian central neuron, the rat retinal ganglion cell. Blockade of the physiological response suggests that toxin F may be a useful probe for the isolation of a functional nicotinic receptor from the mammalian CNS.

The block produced by toxin F was only partially reversible. Possible explanations for the lack of full recovery from toxin F might be that at least some of the nicotinic channels were essentially irreversibly inactivated by high-affinity binding of the toxin or that toxin-channel complexes underwent endocytosis into the cell. Following washout of the toxin from the bath, either new channels may have been inserted into the membrane or channels with lower-affinity binding may have been able to shed the toxin (Loring and Zigmond 1987). Whatever the mechanism, the partial re-

versibility of blockade by toxin F resulted in a reliable method of observing single nicotinic channels during whole-cell recordings. Other techniques were used to record discrete acetylcholine channels, including prolonged superfusion in high agonist concentrations to produce partial desensitization during whole-cell recordings (Fenwick et al. 1982), and brief application of low agonist concentrations during recordings from excised membrane patches in an attempt to activate single channels. Although single channels were resolved by these techniques, the yield of data was small compared to that generated during the recovery phase following toxin F block. The unitary conductance of single nicotinic channels of 48 pS at 35° C obtained in this study is comparable to that observed in cultured neurons from chick ciliary ganglia (38–42 pS at 30° C; Ogden et al. 1984) and in other neuronal-like cells such as bovine adrenal chromaffin cells (44 pS at ~ 20° C; Fenwick et al. 1982). At lower temperatures multiple conductance states have been reported for muscle nicotinic channels (10, 25 and 35 pS at 5° C in rat myoballs; Hamill and Sakmann 1981). We have found no evidence for multiple distinct conductances in central neural nicotinic channels during recordings from individual retinal ganglion cells, but all of our data were collected above 32° C.

In summary, the nicotinic receptors on mammalian retinal ganglion cells in the central nervous system appear to have a pharmacology similar to the receptors found on autonomic ganglion cells in the peripheral nervous system but different from those at the neuromuscular junction. This finding strongly suggests that this central neural nicotinic receptor has greater homology to that of the peripheral nervous system than to that of muscle. Recent molecular biology studies support the conclusion that neural nicotinic receptors and neuromuscular junction receptors are distinct (Boulter et al. 1986). The data presented here are the first to directly characterize the nicotinic response in an isolated mammalian central neuron. This information provides a pharmacologic profile which will be necessary for the proper identification of at least one nicotinic receptor from the mammalian central nervous system when it is biochemically purified and its gene cloned. Moreover, further studies of these electrical responses are likely to lead to a better understanding of the function of nicotinic receptors in the mammalian brain.

*Acknowledgements.* We thank Matthew Frosch for helpful suggestions and computer programs, Micheal Phillips and Kimberly Weber for technical assistance, and David Tauck for comments on the manuscript. We are also grateful to Dinah Sah and Richard Zigmond for allowing us to read their manuscript on toxin F while it was in press. This work was supported by NIH (USA) grants EY05477, NS00879, NS12651, NS22472, and NS07264.

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Received January 30, 1987/Accepted May 4, 1987