

Voltage Clamp Study of Fast Excitatory Synaptic Currents in Bullfrog Sympathetic Ganglion Cells

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ABSTRACT Excitatory postsynaptic currents (EPSCs) have been studied in voltage-clamped bullfrog sympathetic ganglion B cells. The EPSC was small, rose to a peak within 1–3 ms, and then decayed exponentially over most of its time-course. For 36 cells at -50 mV (21 – 23°C), peak EPSC size was -6.5 ± 3.5 nA (mean \pm SD), and the mean decay time constant τ was 5.3 ± 0.9 ms. τ showed a small negative voltage dependence, which appeared independent of temperature, over the range -90 to -30 mV; the coefficient of voltage dependence was -0.0039 ± 0.0014 mV^{-1} ($n = 29$). The peak current-voltage relationship was linear between -120 and -30 mV but often deviated from linearity at more positive potentials. The reversal potential determined by interpolation was ~ -5 mV. EPSC decay τ had a $Q_{10} = 3$. The commonly used cholinesterase inhibitors, neostigmine and physostigmine, exhibited complex actions at the ganglia. Neostigmine (1×10^{-5} M) produced a time-dependent slowing of EPSC decay without consistent change in EPSC size. In addition, the decay phase often deviated from a single exponential function, although it retained its negative voltage dependence. With 1×10^{-6} M physostigmine, EPSC decay was slowed but the decay phase remained exponential. At higher concentrations of physostigmine, EPSC decay was markedly prolonged and was composed of at least two decay components. High concentrations of atropine (10^{-5} to 10^{-4} M) produced complex alterations in EPSC decay, creating two or more exponential components; one decay component was faster and the other was slower than that observed in untreated cells. These results suggest that the time-course of ganglionic EPSC decay is primarily determined by the kinetics of the receptor-channel complex rather than hydrolysis or diffusion of transmitter away from the postsynaptic receptors.

INTRODUCTION

Voltage clamp analysis of synaptic currents has provided important insight into the kinetics of transmitter-receptor interactions especially at vertebrate

and invertebrate neuromuscular junctions (Takeuchi and Takeuchi, 1959; Kordas, 1969, 1972 *a,b*; Magleby and Stevens, 1972 *a,b*; Anderson and Stevens, 1973; Dudel, 1974, 1977; Gage and McBurney, 1975; Crawford and McBurney, 1976; Anderson et al., 1978; Onodera and Takeuchi, 1978, 1979). Many of the conclusions drawn from these studies have been substantially strengthened by analysis of agonist-induced membrane noise (Katz and Miledi, 1972; Anderson and Stevens, 1973). Similar studies have been done recently on invertebrate neuronal synapses (Adams et al., 1976; Llinas et al., 1974; Ascher et al., 1978 *a,b*). However, except for the recent report by Kuba and Nishi (1979), which appeared while this article was in preparation, no comparable studies have been published utilizing a vertebrate neuronal synapse.

In this paper we report the results of a voltage clamp study of the synaptic current underlying the fast nicotinic, excitatory postsynaptic potential in B cells of the bullfrog sympathetic ganglion. The large B cells have an anatomically simple input-output relationship; one or two presynaptic fibers wrap around the axon hillock and branch into multiple synaptic endings on the soma (Nishi and Koketsu, 1960; Weitsen and Weight, 1977). Since there is no intervening dendritic membrane, one can record the synaptic response directly at the soma.

Many of the results obtained in this study are qualitatively in agreement with those recently reported by Kuba and Nishi (1979) although important quantitative differences are apparent. In brief, our results demonstrate that the fast excitatory postsynaptic current (EPSC) rises rapidly to a peak value then decays exponentially. The decay time-course has a small negative voltage dependence and a Q_{10} of ~ 3 . The EPSC peak current-voltage relationship is approximately linear at negative voltages but often flattens at positive voltages. Further, in the presence of high concentrations of atropine, the EPSC decay time-course becomes complex and can no longer be described as a single exponential function. These results suggest that the time-course of ganglionic EPSC decay is primarily determined by the kinetics of the receptor-channel complex rather than hydrolysis or diffusion of transmitter away from the postsynaptic receptors. Preliminary accounts of some of these observations has been presented previously (MacDermott et al., 1978 *a,b*; 1979).

METHODS

All experiments described in this report were done *in vitro* on cells in the IX and X ganglia from the paravertebral sympathetic chain of the bullfrog, *Rana catesbiana*. The sympathetic chain from the 5th to the 10th ganglia and the IX and X spinal nerves were excised and placed in a small glass-bottomed lucite dish for recording. To facilitate electrode penetration into individual neurons, several layers of connective tissue lying close to the cell bodies were removed by careful dissection. The preparation was then mounted on the stage of a compound microscope, so that individual ganglion cells could be seen under bright field illumination at $\times 150$ – 200 . In most preparations, the largest cell bodies (30–60- μm diameter) could be readily localized and impaled

with two microelectrodes under visual control. There are two postganglionic cell types in amphibian sympathetic ganglia, B cells and C cells; in addition, there are a few small intensely fluorescent cells (Weight and Weitsen, 1977). The present study was confined to B cells by placing the bipolar platinum stimulating electrodes between the sixth and eighth ganglia. This limited the stimulation to preganglionic B fibers which innervate B cells exclusively (Nishi et al., 1965). Furthermore, identification of cell type was readily confirmed by considering cell size, threshold, and conduction velocity.

Most of the experiments were performed using preparations maintained in a HEPES (*N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid)-buffered solution (millimolar: NaCl 117, KCl 2.5, CaCl₂ 1.8, HEPES 1.0, pH = 7.3). A few experiments were done in a phosphate-buffered solution to ensure that the results obtained were independent of the particular buffer used. No difference in results was observed in those preparations. The volume of the solution bathing the preparation was \approx 3 ml. Bath temperature was controlled by circulating ethylene glycol at a desired temperature through the stage of the microscope. Temperature remained stable after an equilibrium period of 10–20 min, and could be varied from 10 to 27°C.

Atropine sulfate (Merck Chemical Div., Merck & Co., Inc., Rahway, N.J.), neostigmine bromide (Sigma Chemical Co., St. Louis, Mo.), and physostigmine sulfate (Merck) were used in a few experiments. Stock solutions of each drug were made fresh daily and diluted just prior to use.

Individual ganglion cells were voltage-clamped using a two-electrode, point voltage-clamp system similar to that described by Dionne and Stevens (1975). Clamp gain was a nonlinear function of frequency, equaling the open loop gain of the output stage ($\sim 10^6$ V/V) at low frequencies and being rolled off at higher frequencies with an adjustable feedback resistance capacitance network to maximize response time without ringing. With this system, cell membrane potential could be held over the range -120 to $+60$ mV, although most experiments were confined to voltages between -90 and $+30$ mV. Commonly, both the voltage and current electrodes were filled with 3 M KCl, although in a few experiments 0.6 M K₂SO₄ current electrodes were used with no obvious difference in the results. To facilitate voltage control and reduce background noise, voltage and current electrodes were chosen with resistance of 6–20 M Ω . The majority of cells impaled with two electrodes had resting potentials (unclamped) between -20 and -40 mV. Before the cells were voltage-clamped, preganglionic stimulation produced either a subthreshold EPSP or an action potential. Under voltage-clamp conditions, only EPSCs were recorded except in a few instances when additional rapid current forms were superimposed on the EPSC. These very rapid current spikes may have been associated with action potentials initiated at distal portions of the axon hillock and propagated into the soma. This suggested that voltage control did not always extend as far as the most distant synaptic boutons in these cells. Data were collected only from those cells in which other currents did not interfere with the EPSC.

Three criteria were used to determine acceptability of the data: first, a stable holding current while the cell was clamped at a designated voltage; second, DC drift of no more than 5 mV from base line at the end of the experiment; third, a peak voltage deviation of $< 0.5\%$ of the driving force with driving force defined as the membrane potential minus the EPSC reversal potential (~ -5 mV). Typically, at -50 mV membrane potential, the peak voltage deviation was < 100 μ V and returned to command setting before the peak EPSC. The present study represents data collected from at least 200 cells from more than 60 ganglion preparations.

EPSCs were elicited at a constant stimulation frequency (0.3–0.4 Hz) and individual currents were usually filtered at 5 or 2 kHz. The current and voltage data were digitized and stored by a PDP 11/34 computer (Digital Equipment Corp. Marlboro, Mass.) For the analysis, an averaged value of the holding current just prior to the stimulus artifact served as the zero current base line for each synaptic response. Individual EPSCs recorded at the same voltage were aligned by peaks and averaged to increase the signal-to-noise ratio. All of the results reported here were derived from averaged EPSCs (4–20 responses). However, under all the conditions used in these studies, each individual EPSC was qualitatively the same as the averaged EPSC. Each peak current value was the average of three points around the peak: these values in turn were used to obtain the total peak EPSC average. Data that are the average of values obtained from several cells are consistently presented as mean \pm standard deviation.

RESULTS

Excitatory Postsynaptic Current

EPSCs were recorded in voltage-clamped sympathetic postganglionic cells following preganglionic stimulation. The EPSC rose to a peak value within 1–3 ms and decayed approximately exponentially. The peak amplitude was small, e.g., -6.5 ± 3.5 nA at 21–23°C in 36 cells voltage-clamped to -50 mV. Averaged currents in Fig. 1 illustrate the amplitude and time-course of an EPSC at different values of membrane potential. The exponential nature of EPSC decay is indicated by the linearity of the data plotted semilogarithmically above the responses; a linear least squares regression line gave an estimate of decay time-constant τ . This analysis indicated that most of the EPSC decay phase could be described empirically by the single exponential expression:

$$\text{EPSC}(t) = \text{EPSC}(0) \exp(-t/\tau).$$

In the example of Fig. 1 $\tau = 5.2$ ms at -50 mV. The average value of τ at -50 mV was 5.3 ± 0.9 ms (36 cells at 21–23°C).

A small deviation from the single exponential was observed in some cells in the latter portion of the EPSC decay. To avoid the influence of this tail component on estimates of the decay time-constant, the analysis was limited to the current decay phase between ≈ 80 and 20% of the peak current.

Peak EPSC Amplitude Was a Function of Membrane Potential

Peak EPSC amplitude varied as a function of membrane voltage. The reversal potential for the EPSC determined by the interpolation was -5.0 ± 1.2 mV ($n = 25$). Fig. 2 shows the relationship between peak EPSC and voltage in two cells over the range -100 to $+50$ mV. These examples illustrate the variable curvature of the peak current-voltage relationship that was quite linear in some cells and markedly curved in others. Although currents from these two cells exhibited greatly different peak values at a given voltage and were measured at different temperatures, the curvature of the I - V relation studied in 25 cells did not vary consistently with these two parameters. Rather the

extent of any deviation from a simple linear relationship, especially at voltages less negative than -20 mV, varied from preparation to preparation; it was apparent in $\sim 50\%$ of the cells studied. Similar observations were described recently by Kuba and Nishi (1979).

EPSC Decay τ Had a Small Negative Voltage Dependence

Kuba and Nishi (1979) reported that EPSC decay was voltage dependent such that the half-decay time increased with hyperpolarization in an approx-

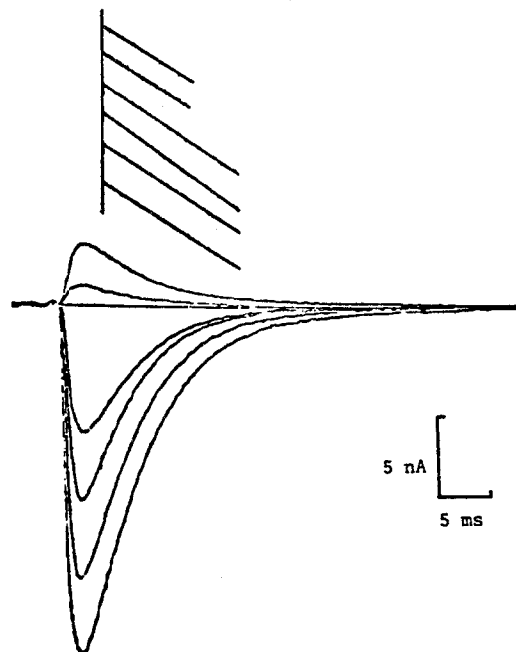


FIGURE 1. Averaged EPSCs recorded from a ganglion cell voltage-clamped to $+20$, $+10$, -30 , -50 , -75 , and -90 mV (top to bottom). Each trace represents the average of at least five individual currents. Above the records, the decay phase of the currents is plotted semilogarithmically as a function of time; the line corresponding to the EPSC decay obtained at $+20$ being uppermost. For the EPSCs obtained at positive voltages, the semilogarithmic plots were limited between 80 and 20% of decay phase because the final portion of the decay appeared nonexponential. In contrast, for those EPSCs obtained at negative potentials, the decay was adequately described by a single exponential function over most of their time-course. The temperature was 22°C .

imately exponential manner. We also investigated the voltage dependence of EPSC decay. At membrane potentials more negative than -30 mV, our results qualitatively agree with those of Kuba and Nishi; however, the behavior of EPSC decay τ at more positive membrane potentials was variable.

The time constants for the averaged EPSCs recorded from two different cells are plotted in Fig. 3. These data illustrate the two general types of

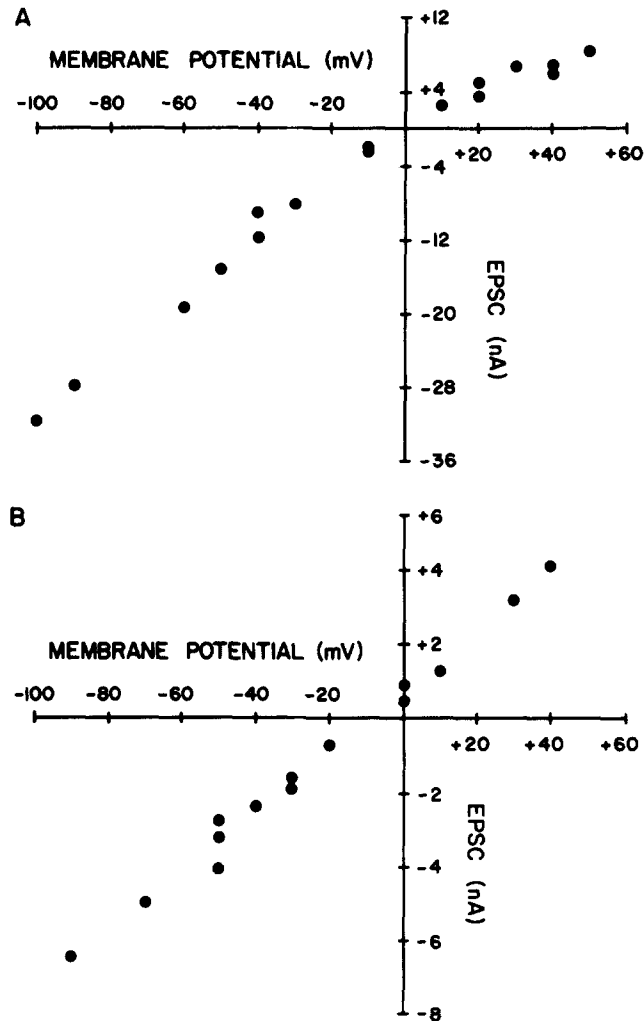


FIGURE 2. Peak EPSC-voltage relationship. The peak $I-V$ curve was used to estimate the reversal potential of the fast EPSC process by interpolation. The $I-V$ curve was linear in about half the cells studied (B) but was characteristically curved (A) at positive potentials in other cells. Although these data were obtained at two temperatures ($\sim 21^\circ\text{C}$ in A, $\sim 13^\circ\text{C}$ in B) and the EPSC values in A were among the largest recorded, neither temperature nor the magnitude of the currents was correlated with the curvature of the $I-V$ relation.

changes in τ which occurred with voltage. In Fig. 3 A the decay rate varied smoothly with voltage in a reversible manner independent of the cell's previous voltage history. By contrast, Fig. 3 B shows a decay rate which increased nonreversibly after the cell was held at depolarized membrane potentials. The numbers above the individual τ values in Fig. 3 indicate the sequence in which the EPSCs were recorded. The order in which the data were recorded in Fig. 3 B is typical of the majority of the experiments. Due to the frequently

irreversible slowing of the decay which occurred during and following clamp episodes at positive membrane potentials, the voltage sensitivity of decay was estimated from currents recorded when the history of the membrane potential was always more negative than -30 mV.

In the range of membrane potentials between -30 and -90 mV, τ showed a negative dependence on voltage. The τ vs. voltage relation could be fit by the equation

$$\tau(V) = a \exp((AV),$$

where a and A are constants. The average value of the voltage-sensitive

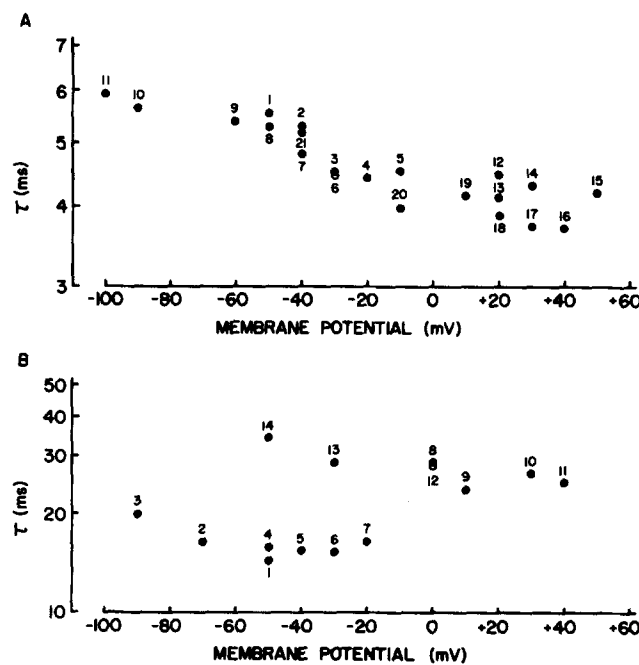


FIGURE 3. Semilogarithmic plot showing the effect of membrane potential on EPSC decay τ for two different ganglion cells. Data from the same two cells used for Fig. 2. The numbers above the data points indicate the sequence in which averaged EPSCs were recorded.

coefficient A was -0.0039 ± 0.0017 mV^{-1} ($n = 15$) at 14 – 18°C . We also tested whether the magnitude of the voltage dependence of τ was a function of temperature. In 29 experiments over a temperature range of 13 – 27°C the voltage-dependence coefficient did not show any consistent temperature dependence. The average value of A was -0.0037 ± 0.0014 mV^{-1} over this extended temperature range.

The Analysis of Decay Was Not Limited by the Small Size of the EPSC

Under normal conditions the size of the largest EPSC was small, and we were concerned that this might limit an accurate estimation of the decay τ .

Consequently, we have analyzed the decay τ in other experiments in which EPSC size was enhanced by increasing evoked transmitter release. Brief trains of high-frequency stimulation (4–70 Hz) were applied to the preganglionic nerve trunk. This allowed comparison of τ values of EPSC decay just before and then following a brief period of high frequency stimulation. Under these conditions peak EPSC size was increased two-to-three fold over that seen before the train, as would be expected due to enhanced transmitter release by posttetanic potentiation (Tashiro et al., 1976). The results from eight similar experiments are summarized in Table I, where the averaged EPSC recorded just before and immediately following a ~ 10 -s train of high-frequency preganglionic stimulation is presented. Inspection of these results shows that there

TABLE I
VALUES OF τ WERE INDEPENDENT OF EPSC SIZE AND
AMOUNT OF ACETYLCHOLINE RELEASED

Experiment	Membrane potential	Frequency of preganglionic stimulation	τ_1 *	EPSC ₁ size*	τ_2 †	EPSC ₂ size†
			ms	nA	ms	nA
A	-40	70	5.2	-6.3	5.5	-11.2
B	-30	70	4.4	-2.3	4.8	-4.1
C	-40	40	5.1	-9.0	5.1	-16.5
D	-50	4	8.6	-8.7	10.1	-13.2
E	-50	20	11.1	-1.3	13.4	-3.3
F	-50	20	13.7	-2.8	14.6	-8.1
G	-50	10	13.0	-4.3	12.0	-8.6
H	-50	36	9.0	-4.1	8.8	-14.9

* τ_1 and EPSC₁ size represent values obtained prior to ~ 10 -s period of high-frequency stimulation. Frequency was 0.3–0.4 Hz.

† τ_2 and EPSC₂ size represent values obtained immediately following the ~ 10 -s period of high-frequency stimulation.

was no significant change in the value of the EPSC decay time-constant although the peak current after stimulation was many times larger than the control. These experiments demonstrated that estimates of τ were independent of the EPSC size and also the amount of transmitter released.

Fast EPSC Decay Was Not Influenced by the Muscarinic Action of Acetylcholine

Amphibian sympathetic ganglion cells produce a slow excitatory postsynaptic potential (the slow EPSP) in addition to the fast EPSP. The muscarinic slow EPSP has been attributed to a decrease in membrane conductance to K⁺ ions that allows the resting Na⁺ conductance to shift the membrane potential to more depolarized values (Weight and Votava, 1970). Any base-line conductance change associated with the slow EPSP might alter the time-course of synaptic currents recorded in these studies. As a first step to avoid such complications the fast EPSCs described in this report were elicited at a frequency of 0.3–0.4 Hz. Tosaka et al. (1968) have reported that the minimum frequency required to elicit a measurable slow EPSP was ≈ 2 Hz, nearly 10-

fold greater. In addition, we analyzed EPSCs obtained in the presence of 10^{-6} to 10^{-7} M atropine, a muscarinic blocking agent. In this concentration range, atropine eliminates the slow EPSP (Tosaka et al., 1968). Fast EPSCs from different cells in the same ganglion preparation were recorded in the presence and absence of low concentrations of atropine. The EPSC recorded at all voltages (-90 to $+30$ mV) appeared similar in the presence and absence of the drug. In addition, the peak current-voltage relationship and the EPSC reversal potential were not changed. The data in Table II indicate that τ was not significantly different when EPSCs were recorded in the presence and absence of low concentrations of atropine at -50 mV.

TABLE II
LOW CONCENTRATIONS OF ATROPINE HAD NO
SIGNIFICANT EFFECT ON τ OF EPSCs RECORDED AT -50 mV

Treatment	Temperature	τ	n^*
	$^{\circ}\text{C}$	ms	
Control	14	17.9 ± 2.4	4
10^{-8} M Atropine	14	15.0 ± 5.0	2
Control	15	11.4 ± 2.3	2
5×10^{-7} M Atropine	15	16.1 ± 4.3	2
Control	18	11.0 ± 4.7	2
10^{-7} M Atropine	18	7.2 ± 0.1	2
Control	27	3.5 ± 0	2
10^{-7} M Atropine	27	3.4 ± 0.4	2

Results are mean \pm SD, $n = 4$; mean \pm range, $n = 2$.

* n indicates the number of cells/group.

Neostigmine and Physostigmine Produce Complex Alterations of EPSC Decay

The voltage dependence of muscle endplate current (EPC) and miniature endplate current (MEPC) decay remains after treatment with neostigmine (Magleby and Stevens, 1972 *a*; Kordas, 1972 *b*; Gage and McBurney, 1975). This observation suggested that the voltage dependence of decay was not due to a voltage-dependent rate of acetylcholine hydrolysis. We have analyzed ganglion EPSCs following treatment with 1×10^{-5} M neostigmine in an attempt to determine whether the voltage dependence of decay in the ganglion preparation is mediated by the cholinesterase. Following neostigmine treatment EPSC size was not significantly changed but the time-course of decay was markedly prolonged, and this increased progressively with time. Further, in the presence of neostigmine the EPSC decay time-course appeared to deviate from a single exponential function. The deviation was evident after a 15-min exposure to neostigmine and became more exaggerated with time. The prolongation of decay time-course is illustrated in Fig. 4 which shows averaged EPSCs recorded at -90 mV in four different cells from the same preparation ($\sim 15^{\circ}\text{C}$). Example A is a current obtained in a control cell, and B-D are

currents obtained after ~ 15 , 30, and 50 min of exposure to 1×10^{-5} M neostigmine. Due to the complex shape of the decay time-course, the EPSC decay phase between 80 and 20% of peak was described by half-decay time. These $t_{1/2}$ values were used to estimate the voltage dependence of decay in the neostigmine-treated cells. In four cells the voltage-dependence coefficient over the restricted voltage range -90 to -30 mV was -0.0042 ± 0.0030 mV $^{-1}$. The peak EPSC-voltage relationship and reversal potential were similar in neostigmine-treated and untreated cells.

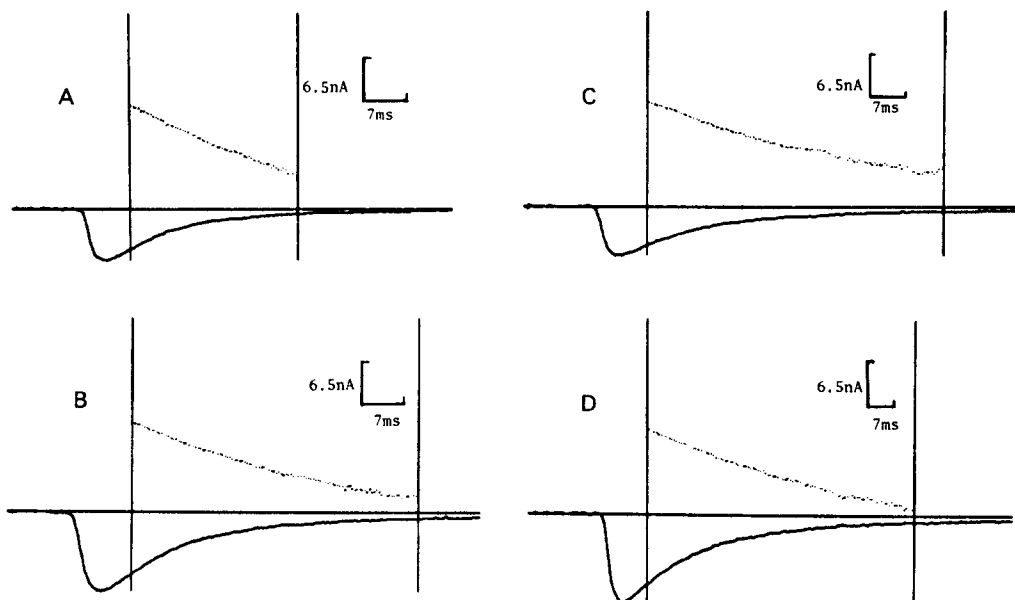


FIGURE 4. Averaged EPSCs from different cells in one ganglion preparation illustrating the progressive prolongation of current decay time-course during exposure to 1×10^{-5} M neostigmine. EPSCs were obtained at -90 mV and 15°C . Logarithmic current values are presented above each current trace. (A) An averaged EPSC obtained in the absence of neostigmine. (B-D) averaged EPSCs recorded after 15, 30, and 50 min of exposure to neostigmine.

Although the peak current-voltage relationship was unchanged in neostigmine, the current decay was slowed and nonexponential while remaining voltage-dependent. Additional experiments were done to analyze the properties of EPSC decay following esterase inhibition by physostigmine. Exposure to physostigmine also altered the time-course of EPSC decay with variable effects on EPSC size. However, the effects differed markedly from those of neostigmine. At $\sim 10^{-6}$ M physostigmine, EPSC decay was slowed, but could still be fitted by a single exponential function of time in most cells (18 – 23°C). In some preparations EPSC size was increased, while in other preparations, there was no obvious change in current amplitude. In the presence of 1 – 5×10^{-5} M physostigmine, EPSC decay became very much slowed and multiex-

ponential. The size of the EPSC was not increased and often was depressed. At high concentrations, $\sim 10^{-4}$ M, ganglionic transmission was blocked in most cells, but when present, the EPSCs were very small and the decay time-course was greatly prolonged and consisted of at least three exponential components. Examples of currents illustrating the alteration of EPSC decay by physostigmine in different voltage-clamped cells from the same ganglion preparation are presented in Fig. 5. Each record is an averaged EPSC obtained

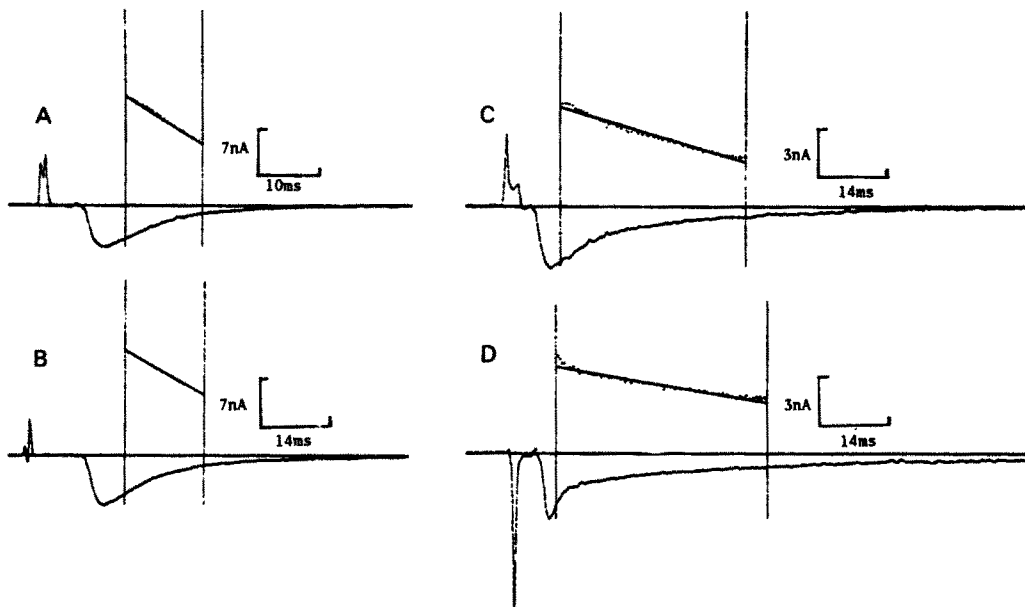


FIGURE 5. Alteration in the EPSC decay by physostigmine. Each current recorded represents an averaged EPSC from different cells in the same ganglion preparation recorded at -70 mV and 19.5°C . Above each current trace, a least squares regression line fitted to the logarithmic of the absolute current values is shown. Example A is an EPSC recorded from a control cell with τ equal to 8.3 ms. Examples B-D represent currents obtained from three different cells in 1×10^{-6} M, 1×10^{-5} M, and 5×10^{-5} M physostigmine, respectively. In example B, EPSC decay was prolonged but could still be fit adequately as a single exponential function with τ equal to 11.6 ms. In examples C and D, the decay phase of the EPSC was markedly slowed and could be adequately fit only as the sum of the two exponential functions. For C, the two time constants were 6.6 and 33.8 ms. For D, the two time constants were 2.1 and 46.8 ms.

at -70 mV and 19.5°C . Record A is a control EPSC with τ equal to 8.3 ms. Records B-D show currents obtained from three different cells exposed to 1×10^{-6} M, 1×10^{-5} M, and 5×10^{-5} M physostigmine, respectively. In record B, EPSC decay was prolonged but could still be fit adequately as a single exponential function with τ equal to 11.6 ms. In examples C and D, the EPSC decay phase was markedly prolonged and could be adequately fit only as the sum of two exponential functions. For record C, the two time constants were

6.6 and 33.8 ms, and for record D they were 2.1 and 46.8 ms. Previously, it has been suggested that physostigmine enhanced the slow EPSP (McIssac and Albrecht, 1975). Therefore, it might be suggested that the slow tail component recorded in physostigmine resulted from an enhanced muscarinic response. However, the slow tail component of EPSC decay in physostigmine-treated cells remained after exposure to 2×10^{-6} M atropine. The results obtained in one of these experiments are summarized in Table III. These observations demonstrate that the prolongation of the EPSC and alteration in the decay time-course do not result from an accentuation of muscarinic activation. Although the EPSC decay time-course was markedly altered in the presence of physostigmine, the shape of the peak EPSC-voltage relationship and reversal potential did not differ from controls.

TABLE III
 PRESENCE OF TAIL COMPONENT OF EPSC DECAY IN
 PHYSOSTIGMINE IS NOT RELATED TO AN ENHANCED
 MUSCARINIC ACTIVATION IN CELLS VOLTAGE-CLAMPED
 TO -50 mV AT 23°C

Treatment	Cell	τ_1	τ_2	Peak EPSC
		ms	ms	nA
Control	1	3.9	—	-6.0
	2	3.7	—	-2.9
Physostigmine $\sim 10^{-5}$ M	3	4.6	18.2	-3.7
	4	3.4	13.5	-3.2
Physostigmine $\sim 10^{-5}$ M + atropine 2×10^{-6} M	5	3.3	27.5	-2.9
	6	2.0	12.7	-1.3

Our observations indicate that neostigmine and physostigmine have complex postsynaptic actions in the ganglion preparation. Therefore, their usefulness is limited in the determination of the role of acetylcholine hydrolysis in normal ganglionic transmission.

EPSC Decay Had a Q_{10} of ~ 3

Experiments to determine the temperature dependence of EPSC decay were done over a temperature range of 10.5 – 23°C . It was not possible to estimate τ at different temperatures in the same cell as has been done previously in other preparations (Kordas, 1972; Magleby and Stevens, 1972 *b*, Gage and McBurney, 1975), because we were unable to voltage clamp individual ganglion cells for the length of time required to effect a stable change in bath temperature. Consequently, the Q_{10} of τ was estimated using values of the EPSC decay time constant observed at -50 mV from individual cells at different temperatures.

The results from 53 cells in 16 ganglion preparations are summarized in Fig. 6 where natural logarithm τ values were plotted as a function of temperature. The estimated Q_{10} of τ was 3. This marked influence of temper-

ature on the EPSC decay time-course is illustrated in the example shown in Fig. 7. These averaged EPSCs obtained from two different cells in the same ganglion preparation had decay τ 's of 21.4 ms (10.5°C) and 5.1 ms (22°C).

Lowering the temperature also increased the rise time of the EPSC and decreased peak EPSC amplitude. Both effects are evident in Fig. 7. The Q_{10} of the change in peak EPSC amplitude with temperature using values from 53 cells clamped at -50 mV over a temperature range between 10.5 and 23°C was 1.7. However, because variability of EPSC amplitudes among individual cells at all temperatures, we have not quantified this change in any more detail. Nor have we attempted to quantitate the alteration in rise time with temperature although it appeared to increase uniformly as temperature decreased. Both these changes were described by Kuba and Nishi (1979) and may reflect the previously reported temperature dependence of presynaptic (i.e., release characteristics, Katz and Miledi, 1965) and postsynaptic (i.e., activation of the receptor channel complex, Gage and McBurney, 1975) functions.

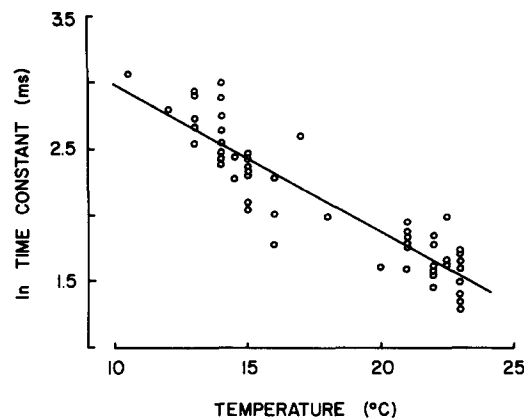


FIGURE 6. Logarithm of EPSC decay τ values from 53 cells voltage-clamped at -50 mV as a function of temperature. The slope of the linear regression line drawn through the data points indicates the strong temperature dependence of EPSC decay τ which has a Q_{10} of 3.

EPSC Decay Became Complex in High Concentrations of Atropine

In the studies described previously concerning the possible influence of muscarinic activation on the EPSC decay, we noted that atropine in low concentration (10^{-7} to 10^{-6} M) did not affect EPSC decay. At higher concentrations however, atropine produced complex alterations in the EPSC decay. The decay in the presence of 10^{-5} to 10^{-4} M atropine was similar to that of muscle EPCs recorded after exposure to local anesthetics (Beam, 1976 *a,b*; Ruff, 1977).

Atropine had two obvious effects on the ganglion synaptic currents: it reduced peak current amplitude and it split the decay phase into two or more exponential components. The change in the EPSC by atropine is illustrated

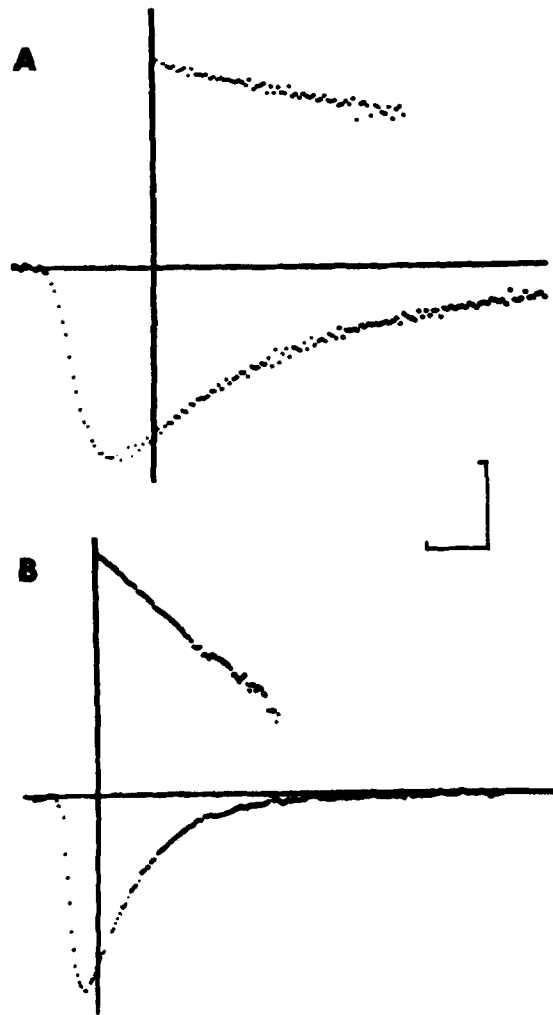


FIGURE 7. Averaged EPSCs recorded from two cells in the same ganglion with the natural logarithm of the current values plotted above each. Record A was at 10.5°C , -50 mV , and had a τ of 21.4 ms . Record B was at 22°C , -50 mV , and had a τ of 5.1 ms . The vertical calibration bar equals 2 nA for current (A) and 8 nA for current (B). The horizontal calibration equals 5 ms .

in Fig. 8. In this figure, averaged EPSCs obtained at -90 mV from an untreated cell may be compared to those obtained from two cells exposed to atropine ($3 \times 10^{-5}\text{ M}$ in part B and $7 \times 10^{-5}\text{ M}$ in C). The decay of the EPSC in the absence of atropine (Fig. 8 A) had a single exponential time-course with a τ of 6 ms . In contrast, the EPSCs shown in Fig. 8 B and C did not have single component decays. The individual components of the decay phase of these atropine-treated cells were estimated with the exponential peeling process described by Beam (1976 *a*). Fig. 8 B had a three-component decay of

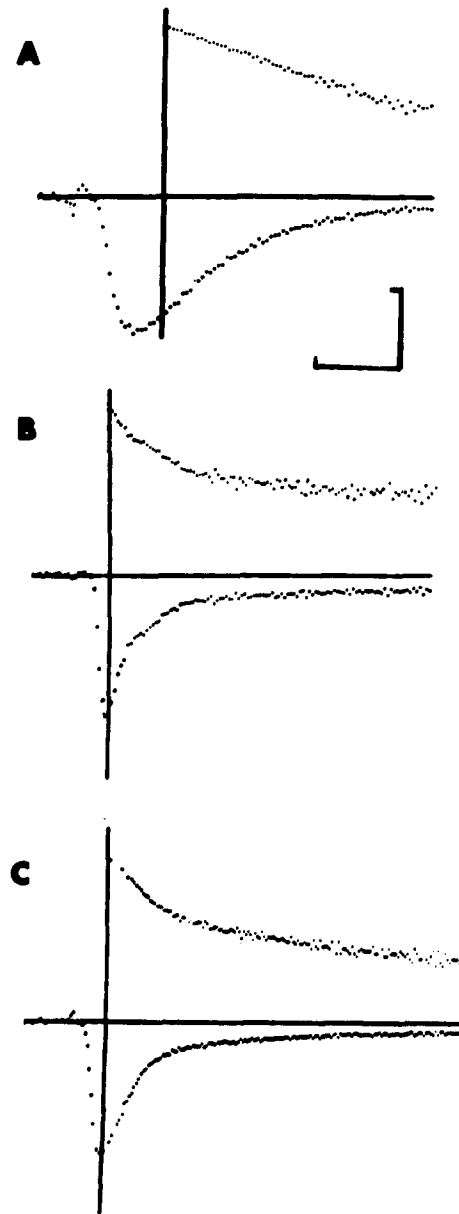


FIGURE 8. Alteration in EPSC decay by high concentrations of atropine. Averaged EPSCs recorded at -90 mV from three different cells at $\sim 18^{\circ}\text{C}$. (A) Control obtained in the absence of atropine. (B) Series of EPSCs obtained from another cell in the presence of 3×10^{-5} M atropine. (C) Third series obtained in the presence of 7×10^{-5} M atropine. The \ln current values are plotted above the falling phase of the EPSC in all three examples. The horizontal calibration bar equals 5 ms, the vertical calibration bar equals 5 nA for A, 1.7 nA for B, and 1.2 nA for C.

which the intermediate and slow phase had τ 's of 3 and 48 ms. We were unable to evaluate the time constant of the very rapid initial component. Example C in Fig. 8 had a two-component decay with time constants of 2 and 20 ms. Each of the three EPSCs shown were recorded from different ganglion cells at bath temperature of $\sim 18^\circ\text{C}$.

High concentrations of atropine depressed peak current without altering the EPSC reversal potential. This is illustrated in Fig. 9 where the peak EPSC-voltage relationship for a control cell and another cell treated with 7×10^{-5} M atropine from the same ganglion preparation are presented. Although the peak EPSC size in the drug-treated cell was smaller than that of the control, the relationship between current and voltage remained constant indicating that over this voltage range reduction of the EPSC amplitude by atropine appeared independent of membrane potential.

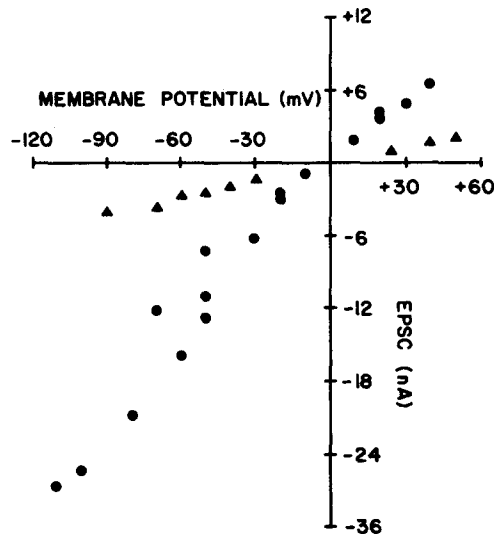


FIGURE 9. The peak current-voltage relationship for two different cells from the same ganglion preparation. A series of EPSCs were recorded in one cell in the presence (▲) and in another cell in the absence (●) of 7×10^{-5} M atropine at 18°C .

The sensitivity to atropine varied among preparations. For instance, in one preparation, 10^{-4} M atropine depressed the currents to such an extent that no analyzable data could be collected. In another ganglion, 10^{-4} M atropine only depressed peak current to about two-thirds of control size. In the present study we have made no attempt to quantify the relationship between concentration and depression of peak current or the number of decay components. Our concern here has been the qualitative observation that atropine could depress peak current and split the EPSC decay into a multicomponent time-course.

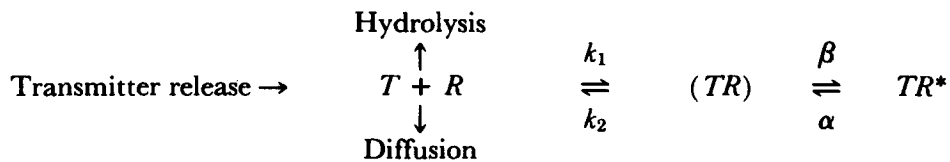
DISCUSSION

The fast EPSC is mediated by cholinergic receptors similar to those at the neuromuscular junction; both are classified as nicotinic. In spite of this, the

possibility that synaptic control mechanisms at these two synaptic membranes may have different properties was raised by earlier observations of pharmacological differences between them. We have found that many of the features of the fast EPSC appear qualitatively similar to the twitch muscle EPC, yet quantitative differences were found in the kinetics of current decay. The ganglionic fast EPSC decayed more slowly, with a more limited voltage dependence, and with a greater variability of decay time-constant than the twitch muscle EPC; its properties were more like those from slow fibers than twitch (Dionne and Parsons, 1978). These differences along with pharmacological modifications of decay time-course implicate receptor kinetics in the control of ganglionic EPSC decay.

We have not identified the cause of the observed shift in the τ vs. voltage relationship which occurred in some cells after voltage clamping to positive values of membrane potential. However, as a matter of speculation, some of the variable effects of voltage on τ may represent secondary changes in channel gating kinetics because of accumulation or depletion of specific ions in the immediate vicinity of the synaptic membrane. Previously, Magleby and Stevens (1972) suggested that changes in both potassium and sodium concentration may affect the EPC decay time-course. This view is strengthened by the recent study of Gage and Van Helden (1979). Also, elevation of external calcium prolongs EPSC decay (Kuba and Nishi, 1979; Connor et al.¹).

The following scheme for synaptic transmission identifies the processes possibly involved at the ganglion in the activation of its nicotinic cholinergic receptors.



Here T represents free transmitter released into the synaptic cleft and R a receptor-channel complex on the postsynaptic membrane. (TR) is an intermediate, nonconducting transmitter-bound complex, and TR^* is the activated, conducting complex. Transmitter may either diffuse out of the cleft, be hydrolysed by acetylcholinesterase, or bind to receptors in the postsynaptic membrane. The transmitter-bound complex is presumed capable of a reversible conformational change between the nonconducting state (TR) and the conducting state TR^* with rate constants β for opening and α for closing.

According to this scheme the decay phase of the EPSC must be controlled either by (a) the time-course of transmitter release, (b) the time-course of transmitter diffusion out of the cleft, (c) the rate of removal of ACh by hydrolysis, or (d) the binding and conformational change kinetics of the receptor-channel complex. There are two extreme competing proposals in case d for what kinetics rates determine the time-course of the transition between R and TR^* . In the first, binding and unbinding of agonist to the receptor are rapid and the rate-limiting process would be the conformational change (Magleby and Stevens, 1972 *a,b*; Anderson and Stevens, 1973). Alternatively, the conformational change might be rapid and the binding steps might be

¹ Connor, E. A., A. B. MacDermott, and R. L. Parsons. Unpublished observations.

rate-limiting (Kordas, 1969; Adams and Sakmann, 1978). An intermediate case in which the apparent decay rate is a function of both the binding and conformational transitions is also possible. Although our data allow us to address cases *a*, *b*, *c*, and *d* our results do not allow us to decide among the two alternates in *d*.

The time-course of transmitter release has not been studied directly in this work. However, we have assumed from the results of previous studies at the neuromuscular junction that the kinetics of release are too rapid to be the normal determinant of EPSC decay (Katz and Miledi, 1965; Barrett and Stevens, 1972; Magleby and Stevens, 1972 *b*). Support for this assumption in the ganglion preparation is derived from our observations that the EPSC decay τ was independent of the modulation of transmitter release by posttetanic potentiation (Table I). Assuming that transmitter release does not mediate normal EPSC decay and given the accuracy of the single exponential description of the time-course, then the observed time-course of decay can be used to estimate the driving function of the synaptic event (Magleby and Stevens, 1972 *b*). The duration of the driving function appears to be no more than a few milliseconds. This function might represent either free cleft [ACh] or the duration of ACh release, depending upon the actual determinant of the fast EPSC decay. In any case, release could not proceed slower than this derived driving function.

The possibility that transmitter diffusion within the synaptic cleft might determine EPSC time-course was addressed through the temperature dependence of decay. The Q_{10} for EPSC decay was ≈ 3 and was similar to that observed at numerous other synapses (Takeuchi and Takeuchi, 1959; Kordas, 1972; Magleby and Stevens, 1972 *a,b*; Gage and McBurney, 1975; Onodera and Takeuchi, 1978, 1979). Kuba and Nishi (1979) reported a value of 6 in this preparation; the reason for the difference in results is unclear because they did not indicate the number of experiments nor the method used to determine the temperature dependence of decay in their study. A Q_{10} near 1 would be expected if the EPSC decay were determined primarily by diffusion of transmitter from the cleft, where a higher Q_{10} value would suggest that the rate-limiting step is a more energy-dependent process. This observation is consistent with the calculations of Eccles and Jaeger (1958) showing that diffusion should be too rapid to account for the duration of transmitter action. Our value of τ equal to ~ 11 ms at 13–16°C (at -50 mV) is much longer than the comparable 0.3 ms the Eccles and Jaeger estimated for diffusion. Therefore, both the high Q_{10} and slow time-course of EPSC decay strongly suggest that some process other than diffusion determines the time-course of EPSC decay.

In an effort to determine whether hydrolysis of ACh by acetylcholinesterase is the normal mechanism governing the decay of the EPSC, we employed the anticholinesterase agents neostigmine and physostigmine. If hydrolysis of ACh proceeded at a rate to account for the decay time-course by reduction of free ACh in the cleft, then poisoning the enzyme should prolong the decay process by changing the normal processes of ACh removal. Following enzyme destruc-

tion, the new mechanism controlling EPSC decay should be slower and possibly might have altered temperature and voltage dependencies; either diffusion or channel kinetics would be viable candidates for this revealed process, but release of transmitter would not.

Both anticholinesterase agents caused the fast EPSC to decay more slowly as would be expected if enzymatic destruction of ACh were the normal rate-limiting step. More importantly, the voltage dependence of EPSC decay in the presence of neostigmine was similar to that normally encountered. A similar voltage dependence of the decay process following neostigmine treatment argues that diffusion is not the new rate-limiting step. However, the possibility exists that the anticholinesterase drugs may interfere with normal channel kinetics as well as inhibit hydrolysis of ACh. Thus, the results are consistent with either hydrolysis or channel kinetics being the normal determinant of EPSC decay, but diffusion is not the control step either before or after treatment by anticholinesterase.

The multicomponent and time-dependent effects of these anticholinesterase drugs deserve careful note. Both neostigmine and physostigmine produced complicated responses which (in one case) became accentuated with the duration of exposure. One cannot convincingly demonstrate that they produce only an inhibition of the esterase at the concentrations used, and, in fact, our results suggest that they may have additional effects on channel kinetics (Dreyer et al., 1976; Stevens, 1978) or release (Riker and Kosay, 1970).

The decay of the EPSC in high concentrations of atropine provides the best evidence we have that the fast EPSC time-course is normally determined by channel kinetics, not ACh hydrolysis. Atropine is a classic muscarinic antagonist; it has no known anticholinesterase effects at the concentrations employed here (Kato et al., 1972). Nevertheless, at the ganglion, atropine produces an alteration of the fast EPSC decay phase which resembles local anesthetic action on nicotinic EPCs (Adams, 1977; Beam, 1976 *a*). The EPSC decay is split into rapid and prolonged decay phases whose amplitudes diminish with increased atropine concentration. This type of phenomenon in muscle EPC has been ascribed to a transient block of open endplate channels (Ruff, 1977). If the same effect occurs here, produced now by atropine, then the normal EPSC decay must be controlled by cholinergic channel kinetics. By contrast, if decay were normally dependent on hydrolysis while atropine had no effect on the behavior of that enzyme, a two-component decay time-course could still be produced if atropine affected release. The necessary effect would be that atropine dramatically increase the amount of transmitter released so that high-concentration saturation effects could produce the rapidly decaying peak current component. Additionally, one should expect such peak currents would be larger than the normal EPSC peak values. Instead, peak EPSC values in atropine were smaller than normal peak values. Furthermore, the slowing of the late EPSC decay component would be difficult to explain by a drug effect on release. Thus, the effects of atropine are most easily explained if the normal determinant of fast EPSC decay is channel kinetics and not hydrolysis. This issue deserves further study. If this

explanation is correct, the effect of atropine on ganglionic fast EPSC decay must be a local anesthetic-like block of open channels.

Conclusion

We have described and quantified some of the basic properties of synaptic currents at ganglionic synapses. Some aspects of the kinetics of the receptor-channel complex in ganglion cells are different from those in the more frequently studied neuromuscular junction. The result of these differences is a slow synaptic current decay that has a relatively small voltage dependence. The implication these conditions have for synaptic transmission at neuronal synapses is interesting to consider. For example, the long duration of synaptic current may continue to produce postsynaptic depolarization following a single action potential. Then, any physiological or pharmacological perturbation that increased EPSC size or slowed its decay could result in a longer synaptic depolarization and possibly induce repetitive firings in a graded fashion following a single orthodromic activation.

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