Synaptic Current at the Rat Ganglionic Synapse and Its Interactions With the Neuronal Voltage-Dependent Currents

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INTRODUCTION

The basic properties of the acetylcholine-gated receptor channel complex have been intensively studied at the ganglionic synapse and the analysis of the synaptic current time course has been important for understanding the mechanism of transmitter action at the postsynaptic membrane (Connor et al. 1983; Derkach et al. 1983; Mathie et al. 1987; Selyanko et al. 1979). A rich body of evidence has consequently been accumulated concerning the decay phase of the excitatory postsynaptic current (EPSC), its sensitivity to membrane potential, temperature, ionic changes and drug treatments; many of the conclusions drawn from these studies were substantially confirmed by single channel analysis (Cull-Candy and Mathie 1986; Derkach et al. 1987; Mathie et al. 1987). We have used the intact and mature rat sympathetic neuron, which is amenable to voltage-clamp recording methods, to analyze the basic biophysical properties of the naturally released ACh at a mammalian neuron. To date, detailed electrophysiological studies of postsynaptic responses to exogenous ACh have been confined to the acutely dissociated or cultured rat sympathetic neuron (Cull-Candy and Mathie 1986; Derkach et al. 1987; Mathie et al. 1987); no comparable information is available on the postsynaptic response to neurally evoked ACh in neurons with functionally intact synaptic connections. Moreover, the foundation for quantitative description of the synaptic current underlying the synaptic potential is lacking, although it is important to understand how postsynaptic neuronal responses are evoked.

In the first section of this paper the postsynaptic responses to spontaneously released ACh quanta [miniature synaptic currents (mEPSC)] and to electrically evoked release (EPSC) are analyzed and described by a mathematical model. With this approach, the ACh currents are well reproduced by analytic functions over a wide range of membrane potential levels; furthermore, the effect of nonsynchronous transmitter release on the characteristics of synaptic events can be examined and conditions can be defined where minimal distortion arises from temporal dispersion in evoked quantal release.

The analytic description of the synaptic current makes it possible to address the important question as to how the natural synaptic activity drives neuron excitability, which is the topic of the second half of this paper. Previous work from this laboratory indicated that the major voltage-dependent conductances of the neuronal membrane can be individually isolated and kinetically characterized (Belluzzi et al. 1985a,b; Belluzzi and Sacchi 1990). Consequently, we presented a five-conductance model of the rat sympathetic neuron, which accurately predicts the cell electrical behavior.
under current-clamp conditions in response to externally applied current pulses (Belluzzi and Sacchi 1991). We show here that the interactions between the ganglionic synaptic current and the neuronal voltage-dependent current components can be correctly described and interpreted by the complete mathematical model of the sympathetic neuron, in the subthreshold range as well as during the action-potential development. This analysis indicates that the properties of the fast transient potassium current, $I_A$, are of prominent interest under these conditions. In fact, $I_A$ channels not only provide the main repolarizing charge during the action potential falling phase (Belluzzi et al. 1985a), but also contribute the only voltage-dependent conductance typically activatable by excitatory synaptic potentials in the $-65/–40\text{ mV}$ voltage range.

**METHODS**

**Dissection and recording**

All experiments were performed on superior cervical ganglia isolated from young rats (100–130 g body weight) during urethane anesthesia (1 g kg$^{-1}$; ip injection) and maintained in vitro at 37°C. After surgery, the animals were killed with an overdose of anesthetic. Ganglia, including pre- and postganglionic nerve trunks, were mounted on the stage of a compound microscope; individual neurons were identified at a magnification of $\times 500$ by using diffraction interference optics and impaled with two independent glass microelectrodes filled with neutralized 4 M KCl and having resistances of 30–40 MΩ. Recordings were obtained under two-electrode voltage-clamp as described previously (Belluzzi et al. 1985a). The preparations were superfused with a medium that was pregassed with 95% O$_2$–5% CO$_2$ to a final pH 7.3 and had the following ionic composition (mM): 136 NaCl, 5.6 KCl, 5 CaCl$_2$, 1.2 MgCl$_2$, 1.2 NaHPO$_4$, 14.3 NaHCO$_3$, and 5.5 glucose. In order to activate the preganglionic input, single current pulses (0.3 ms duration) of variable strength were applied at 0.05–10 Hz to the cervical sympathetic trunk close to the ganglion through a fine suction electrode. The usual protocol was to hold the potential at $–50\text{ mV}$ and to jump to the test potential in the $–10/–120\text{ mV}$ voltage range 10 ms before a supramaximal preganglionic shock was delivered, returning to the holding level 50 ms after the stimulus. Synaptic currents (EPSCs) could be recorded with good control of the membrane potential at any tested voltage; the small residual voltage change of the fast excitatory postsynaptic potential (EPSP), which remained during voltage clamping, amounted to $–2\text{ mV}$ of the original EPSP and no correction was made for it. Single currents were filtered at 5 kHz with an 8-pole Bessel filter, digitized at 10 kHz with a 12-bit analog-to-digital interface (Digitdata 1200A operating pCLAMP software, Axon Instruments) and stored on disk for further analysis. Long-lasting current tracings containing trains of EPSCs and random miniature currents were recorded continuously on FM tape (Ampex PR2200, 0–5 kHz) and subsequently sampled at 10 kHz with a 12-bit digital oscilloscope (VUKO MC-CP, VUKO GmbH, Muelheim, Germany). Data were analyzed with IBM 386 personal computers with pCLAMP and MATLAB 386 (The MathWorks, Natick, MA) software packages. The muscarinic receptor agonist [(±)-muscarnine chloride, Sigma] and antagonist (atropine sulfate, Sigma) were bath applied by exchanging the normal medium with drug-containing medium by means of a continuous rapid perfusion system.

**Analysis of mEPSCs**

mEPSCs were recorded in normal saline at a holding potential negative to $–75\text{ mV}$ (usually $–120\text{ mV}$). mEPSCs were individu-
of power spectra; 2) the analysis in the time domain escapes problems of circular correlation because of truncation of the record; 3) provided \( P(t) \) vanishes (or falls to negligible levels) within a time period shorter than the duration of the event, the late portion of the autocorrelation decays according to the two time constants of the mEPSC, whereas no regions of the power spectrum are unaffected by \( P(t) \). Figure 1 uses an example where release is somewhat asynchronous to illustrate how the contribution of each time constant and of \( P(t) \) to the EPSC autocorrelation (and to the EPSP time course) can be dissected out by autoregressive filtering.\(^1\)

When unitary events could be seen and analyzed, both procedures were used and the results were compared. As discussed in Results, estimates of \( P(t) \) from the two approaches were consistent, although the decay time constant of unitary event waveform was systematically overestimated by the autocorrelation approach.

**Model for the rat sympathetic neuron**

The five-conductance model of the somatic membrane of the mature rat sympathetic neuron, presented in a previous paper (Beluzzi and Sacchi 1991), was used to simulate the neuron behavior in current clamp. Basically, five separate types of voltage-dependent ionic conductances have been individually isolated at 37°C under two-electrode voltage-clamp conditions and kinetically characterized in the framework of the Hodgkin-Huxley scheme. The mathematical functions for the gating activation and inactivation (where present) mechanism of each current, estimated over a wide voltage range, were reported by Belluzzi and Sacchi (1991): text Eqs. 7–11 for the sodium current, \( I_{Na} \); Eqs. 13–16 for the calcium current, \( I_{Ca} \); Eqs. 22–23 for the \( K^+ \) delayed rectifier, \( I_{Kd} \); Eqs. 24–27 for the fast transient \( K^+ \) current, \( I_{Kt} \); Eqs. 29–32 for the \( Ca^{2+} \)-dependent \( K^+ \) current, \( I_{KCa} \).

We report here the values of the constants used in the present computations: \( g_{Na,fast} = 9.28 \mu S/p\text{er neuron} \); \( g_{Na,slow} = 7.43 \mu S; \) \( f_{Na} = 2.09 \times 10^{-5} \text{cm/s} ; \) \( g_{Ca} = 0.44 \mu S; \) \( g_{Ca,fast} = 2.30 \mu S; \) \( g_{Ca,slow} = 0.45 \mu S; \) \( E_{Na} = +40.0 \text{mV}; \) and \( E_{LEAK} = -73.2 \text{mV}. \) The \( K^+ \) equilibrium potential, \( E_K \), is expected to move from the resting value of -93.0 mV because of \( K^+ \) accumulation in the perineuronal space during outward current flow (Belluzzi and Sacchi 1991) and Eq. 18). The mean leakage conductance, \( S_{LEAK} \), and the membrane capacitance, \( C_m \), in our sample proved to be 0.08 \( \mu S \) and 240 \( \text{pF} \), respectively. When the natural electrical response of a neuron was compared with the predictions of the model however, the values for \( C_m \) and \( S_{LEAK} \) used in the simulation were chosen so as to yield the values directly measured in that neuron for cell input resistance and time constant. The \( I_{AMP} \) current component was not included in the present model because, although clearly identified and well-described analytically in the rat sympathetic neuron (Sacchi et al. 1995), it displays activation kinetics much slower than the events here considered; also neglected was the \( R_m \) current, which was not unequivocally detected in our experiments (Belluzzi et al. 1985b) and, according to the literature, displays a maximum total conductance per cell about 100-fold lower than the synaptic conductances here measured and very slow activation time constants: a maximum \( g_{Na} \) of 1–3 \( nS \) per

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1 The main principles underlying this approach can be briefly summarized as follows. Let the waveform of the mEPSC be described by \( w(t) = \exp(-\alpha t) - \exp(-\beta t) \) \( \{ t \geq 0 \} \): it is easily shown that \( w(t) = A_1 \cdot w(t - \tau) + A_2 \cdot w(t - 2\tau) \), where \( \tau \) is an arbitrary time interval, \( A_1 \) and \( A_2 \) are two constants uniquely determined by \( \alpha \), \( \beta \), and \( \tau \). The same equation holds for the autocorrelation (AC) of \( w(t) \), i.e., \( R(t) = A_1 \cdot R(t - \tau) + A_2 \cdot R(t - 2\tau) \) \( \{ t \geq 0 \} \). The AC of the macrocurrent, \( \beta(t) \) (Fig. 1Aa), is obtained by convolving \( R(t) \) (AC of the micrcurrent) and the AC of \( P(t) \). If \( P(t) \) is negligible for \( t > T \), its contribution to \( \beta(t) \) will be negligible for \( t > 2T \). Thus \( \beta(t) = A_1 \cdot \beta(t - \tau) + A_2 \cdot \beta(t - 2\tau) \) \( \{ t \geq 2T \} \), which yields an easily solvable linear equation set to compute \( A_1 \) and \( A_2 \) (a standard autoregressive moving average approach, ARMA).

The rate constants, \( \alpha \) and \( \beta \), and the mEPSC waveform, \( w(t) \), are directly computed from \( A_1 \), \( A_2 \) and \( \tau \). The simpler, equivalent procedure of sequentially fitting the two rate constants on the decay of \( \beta(t) \) was often employed, as illustrated in Fig. 1. In such cases, \( A_1 \) and \( A_2 \) were computed from \( \alpha \) and \( \beta \). Deconvolution of \( w(t) \) into the EPSC waveform is performed by numerically filtering in the time domain by using the coefficients \( \{ 1, -\alpha, -\alpha^2 \} \); this directly yields the estimated \( P(t) \) (Fig. 1B) (-- -- -- ). For autoregressive approaches see, e.g., Fesce 1990.
cell was estimated for the rat sympathetic neuron in culture, whereas the activation-deactivation time constants for putative M-currents were \( \sim 220 \text{ ms} \) in the \(-60/-30 \text{ mV} \) voltage range (Owen et al. 1990).

RESULTS

Synaptic current at the sympathetic ganglionic neuron

QUANTITATIVE ANALYSIS OF THE EVOKED SYNAPTIC MACROCURRENT (EPSC). Supramaximal stimulation of the sympathetic trunk evokes a synchronous and compact synaptic current in the postganglionic neuron, provided that the stimulus is applied close to the caudal pole of the ganglion. Under these conditions the effect of differences in conduction velocity among the several preganglionic fibers innervating each cell (Perri et al. 1970) is minimized and simple-shaped EPSCs of the type shown in Fig. 2 are usually observed. In Fig. 2, each synaptic current is generated 20 ms after imposing a different membrane voltage in the \(-10/100 \text{ mV} \) range. In the \(-10/-30 \text{ mV} \) tracings the initial steps from the \(-50 \text{ mV} \) holding potential evoked transient \( Na^+ \) currents, which are also displayed to be compared with the corresponding synaptic currents; the onset of the delayed \( K^+ \) currents are evident in the \(-10/-20 \text{ mV} \) recordings. They become increasingly large in amplitude and noise, thereby making the EPSCs virtually undetectable at membrane levels positive to \(-10 \text{ mV} \). Thus a clear-cut reversal of the synaptic currents could not be directly achieved. Between \(-20/-125 \text{ mV} \) the peak amplitude of the synaptic current varied linearly with the membrane potential (Fig. 3A); the regression line extrapolates to a reversal potential between \(-12/-19 \text{ mV} \) (mean \(-15.7 \text{ mV}, n = 7 \)), which is a more negative value than usually obtained for the fast EPSC at other ganglionic synapses. For membrane potentials positive to \(-20 \text{ mV} \) the points lay systematically somewhat below the linear fit. This might be partly because of inward rectification by the ACh-evoked current (Fieber and Adams 1991; Mathie et al. 1990), but it is at least partly accounted for by the changes in ionic gradients that are associated with even small ionic flows (especially accumulation of \( K^+ \) ions in the perineuronal space) (Belluzzi and Sacchi 1990).

In all neurons the decay of EPSC was well fit by a single exponential function. The average time constant, \( \tau \), was 7.5 ms at \(-75 \text{ mV} (n = 14) \) and was scarcely voltage-dependent: it decreased exponentially with a constant of 260.4 mV from \(-105/-25 \text{ mV} \) (Fig. 3A). EPSC amplitudes decreased in the average (5 neurons) to 69% of the initial value when \( Ca^{2+} \) concentration in the bath was lowered from the usual 5 to 2 mM; the current decay time constants were also somewhat decreased (by 21% in the average). The voltage dependence of the decay time constant and the estimated reversal potential did not appear to be affected by calcium concentration (Fig. 3B).

NATURE OF THE POSTSYNAPTIC RECEPTOR ACTIVATED BY NATURAL ACh. To correctly model and reproduce evoked synaptic currents, it is important to determine whether or not conductances other than those associated to the nicotinic receptor were activated by nerve-released ACh. In fact, ACh release by the presynaptic nerve terminals is thought to generate in the ganglion a broad series of side effects, in addition to the central role of sustaining the fast excitatory transmission. Autoreceptors were proposed to control evoked transmitter release (see Starke et al. 1989, for a review). The evidence for a physiological role in the ganglion seems to be greatest for the muscarinic autoreceptors, which are postulated to mediate a negative feedback loop that reduces transmitter mobilization (Koelle 1961; Koketsu and Yamada 1982). A similar case was made for presynaptic muscarinic inhibition of ACh release by preganglionic myenteric neurons (Morita et al. 1982; North et al. 1985). Even at rather low extracellular calcium concentrations, a substantial flow of \( Ca^{2+} \) ions occurs through the neuronal nicotinic ACh receptors, which prove to be more permeable to \( Ca^{2+} \) than muscular \( nAChRs \) are (Fieber and Adams 1991). Single-channel current measurements indicate that \( \sim 2-3\% \) of the current is carried by \( Ca^{2+} \) (Decker and Dani 1990), so that the cholinergic \( Ca^{2+} \) signal is expected to participate in many cellular events. The onset of \( Ca^{2+} \)-activated \( K^+ \) conductance was described in bullfrog sympathetic neurons (Tokimasa and North 1984) after ACh application and, similarly, a \( Ca^{2+} \)-dependent \( Cl^- \) conductance directly activated by \( Ca^{2+} \)-influx through nAChRs was demonstrated in rat central neurons (Müller et al. 1992) and bovine chromaffin cells (Vernino et al. 1992). Finally, stimulation of cholinergic preganglionic fibers is also expected to initiate, besides the conventional fast nicotinic EPSP, various types of “slow” postsynaptic responses. The underlying mechanisms are still controversial, but the evidence suggests an effect mediated via atropine-sensitive receptors (for a review see Akasu and Koketsu 1986). We decided therefore to evaluate to what extent these different mechanisms might contaminate the synaptic currents recorded in the experiments.

In this study \( 10^{-6} \text{ M} \) atropine was tested in five neurons and found to have no effect on the amplitude or time-course...
of the synaptic currents (Fig. 4A). Bath application of the muscarinic agonist (muscarine, 1.5 × 10^{-5} M), on the contrary, caused a consistent reduction of the peak EPSC to a mean amplitude of 68% of the control values at all the membrane voltages tested (Fig. 4B). Atropine completely prevented the muscarine induced depression of EPSC (Fig. 4C), suggesting that muscarine at this concentration actually owes effects to binding to muscarinic receptors. The presynaptic inhibitory receptors might not have been activated in the experiment of Fig. 4A by the amount of ACh released by isolated impulses; this feedback mechanism, in fact, appears to operate in the myenteric neurons only at frequencies >1 Hz (Morita et al. 1982). Thus we applied trains of preganglionic stimuli in the 10–100 Hz range and tested atropine action at high levels of ACh emission. The 10-Hz train of Fig. 5B shows the expected rundown of the second and subsequent EPSCs; no additional or residual current, however, is present in the tracings at -30 or -70 mV membrane voltage level, either within or after the train. The 100-Hz trains (Fig. 5, A and C) demonstrate that the depression of the EPSCs during the trains was the same in the presence of atropine as in its absence; this is illustrated by the difference tracings shown in Fig. 5D.

These experiments would suggest that muscarinic inhibitory receptors can be activated by appropriate agonists applied to the bath, but are not actually operated by endogenous, naturally released ACh; moreover, the nicotinic action of ACh is not accompanied by the activation of additional detectable conductances, at the current resolution available in the present experiments. No ACh-activated conductances other than nicotinic were therefore considered in the analysis and modeling of synaptic currents and of the responses of the sympathetic neuron.

**Figure 3.** EPSC null potential and decay time constant. A: peak amplitude of EPSC as a function of membrane potential (top) and voltage dependence of EPSC decay time constant, \( \tau_d \) (bottom) in rat sympathetic neuron at 37°C over -115 to -15 mV voltage range. Each point represents mean of measurements on same group of 7 cells (14 neurons for -75 mV point, \( \nabla \)) bathed in 5 mM Ca^{2+} saline. Vertical bars indicate SE of means. Line in top panel is regression line of EPSC peak amplitude over membrane potential, omitting -15 mV point, which is probably underestimated. Line in bottom panel shows slope corresponding to an e-fold change of \( \tau_d \) in 260.4 mV.

B: effect of external Ca^{2+} concentration on EPSC peak amplitude and decay. Averaged results from 5 neurons taken in 5 mM Ca^{2+} (○) and after changing solution to one containing 2 mM Ca^{2+} (●). In low Ca^{2+} \( \tau_d \) changes by e-fold in 242.1 mV.

**Quantitative Analysis of the Spontaneous Synaptic Microcurrent (mEPSC).** Random release of quanta from the nerve endings of superior cervical ganglia appears to be negligible at rest, but noticeably increases during preganglionic stimulation, even at low frequency. Quantal analyses previously performed in the same preparation under current-clamp conditions (Saccom et al. 1971) clearly demonstrated that these untriggered potentials exhibited the same amplitude and shape as the smallest EPSPs recorded in the neuron during stimulation of a single preganglionic fiber. It can therefore be assumed that the small inward currents observed in Fig. 6A on top and in between the evoked synaptic macrocurrents are true single quantal events (mEPSCs). Despite their small size, mEPSCs were analyzed in five neurons, held between -75 and -120 mV, in which the noise level was low enough to permit individual events to be detected. For each neuron, the mEPSC amplitude distribution and the relation between amplitude and time-to-peak were determined (Fig. 6, C and D). Because during supramaximal preganglionic stimulation random release is expected to occur at all the release sites located on the neuron, different cable attenuations might be a major source of inaccuracy in subsequent analyses of their amplitudes. No significant correlation, however, was seen between rise time and ampli-
FIG. 4. Effect of muscarinic agonist and antagonist. A: peak current amplitude of averaged EPSCs (data from 5 different neurons) is plotted as a function of membrane potential in presence (●) or absence (○) of 10^{-6} M atropine. B: muscarine decreases peak EPSC. Current-voltage relationships for EPSC are constructed in control solution (○) and ~4 min after bath superfusion with 1.5 × 10^{-5} M muscarine (●; n = 2). C: muscarine effect is prevented by atropine. Points indicate EPSC magnitude before (○) and after (●) 10^{-6} M atropine treatment; 1.5 × 10^{-5} M muscarine was then cumulatively added into bath (●). Final control series (●) was recorded after washing preparation for ~20 min. All data from same cell. Each drug effect was followed for 4–13 min before final recordings were performed; holding potential was ~50 mV throughout.

The amplitude of mEPSCs (in Fig. 6D, the regression line—not shown—exhibits a slope not statistically different from zero) or between their rise and decay time constants. This finding argues against the possibility that mEPSCs were sampled from electrotonically distant locations. Consistently, amplitude distributions were generally well fit by smooth, unimodal lognormal distribution functions (Gaussian distributions could also adequately fit many peak amplitude histograms, especially if occasional oversized events were neglected). For each neuron, mEPSCs were selected according to the requirements that the waveform started from and returned back to the baseline, without artifacts or overlaps to other events, and were averaged to obtain a mean trace, by aligning individual events on the last sample point before the current onset. This procedure was preferred to the more conventional alignment of the miniatures on their peak, because the mEPSCs rose abruptly from the baseline and a correct description of the initial current rise was thought to be of some importance in defining the best fitting function (and the underlying kinetic properties of the channels operated by ACh). The average mEPSC amplitudes ranged from 0.14 to 0.58 nA in the different neurons. From this amplitude

FIG. 5. Postganglionic aftereffects of high-frequency preganglionic stimulation. A and C: summated EPSCs evoked in a sympathetic neuron by trains of supramaximal stimuli applied to preganglionic sympathetic trunk at 100 Hz and recorded at either ~30 or ~50 mV in presence (C) or absence (A) of 10^{-6} M atropine. Corresponding difference tracings are shown in D. B: EPSCs elicited in a sympathetic neuron by trains of 9 supramaximal stimuli at 10 Hz, with neuron held at either ~30 or ~70 mV.
FIG. 6. Miniature EPSC (mEPSC) general properties at ganglionic synapse. A: examples of miniature (*) and stimulus-evoked EPSCs recorded from a single sympathetic neuron at −120 mV during preganglionic stimulation at 1 Hz. B: average time course of 93 mEPSCs from a different neuron, recorded at −120 mV, and least-squares fitted by text Eq. 2 (exponentials had time constants $\tau_2 = 0.52$ ms and $\tau_1 = 4.95$ ms). C: frequency histograms of peak amplitude of mEPSCs averaged in B; continuous line superimposed on histograms represents lognormal distribution best fitting data. Coefficient of variation of quantal EPSCs was 0.28; mean baseline noise was 0.098 nA, with a 0.19 nA standard deviation. D: plot of time-to-peak vs. amplitude of a mEPSC sample recorded at −120 mV. There was no significant correlation between rise time and miniature current amplitude.

and the driving force for the EPSC, the peak conductance of the averaged mEPSC could be estimated to attain values in the range 2.36–5.41 nS (mean 4.04 nS, $n = 5$). The average tracings were always well fit by the difference between two exponentials, yielding a mean value of 0.52 ms (range 0.32–0.75 ms) for the current rise time constant and a mean value of 4.51 ms (range 3.39–5.51) for the current fall, at −75 mV. An example of the best fitting curve is shown in Fig. 6B, superimposed on the discrete samples of the average waveform.

TIME COURSE OF EVOKED QUANTAL RELEASE. Simple-shaped and compact EPSCs were used in the characterization of synaptic macrocurrents. The actual synchronization of the quantal emission was further investigated by analyzing the waveform of macrocurrents evoked in neurons where mEPSCs were previously detected, averaged, and fit. The probability of occurrence (expected instantaneous frequency) of unitary events, $P(t)$, after the delivery of the electrical stimulus to the preganglionic sympathetic trunk was obtained by deconvolving the double exponential fit to unitary currents into the macrocurrent waveform recorded in the same cell (see METHODS). In the example illustrated in Fig. 1, the stimulus was deliberately applied at some distance from the ganglion and release of neurotransmitter is clearly split into a variable number of pulses; it is likely that these pulses correspond to activation of synapses from groups of preganglionic fibers with different conduction velocities (Perri et al. 1970). When the stimulus is applied close to the ganglion, the quantal secretion becomes quite synchronous (Fig. 7A) and the probability of release is now almost fully packed under a sharp, bell-shaped, function of time, indicating that desynchronization of release is re-
FIG. 7. Time course of ACh quantal release evoked by preganglionic stimulation. Comparison of time course of quantal release, after delivery of a single shock to preganglionic trunk, as estimated by using either waveform fit to averaged mEPSCs (A) or difference between 2 exponentials with time constants obtained (as illustrated in Fig. 1) from autocorrelation of EPSC (B). In both panels downward solid lines depict EPSC; (⋯⋯), mEPSC obtained either from average of 23 events (A) or from autocorrelation of EPSC (B), (−−−), P(t), depicts estimated release probability density; and upward solid line, F(t), is computed by integration of P(t) and illustrates cumulative release probability. Notice that 2 procedures yield quite consistent estimates of P(t), although mEPSC waveform estimated from autocorrelation has a slower decay (τ1 = 4.71 ms) than averaged mEPSCs (τ1 = 4.43 ms). Notice also that both procedures highlight presence of a tail of enhanced release, indicated by persisting positive slope in F(t), after phase of synchronous release induced by stimulus.

The composition of the macrocurrent was further analyzed with a different procedure, by assuming a two-exponential time course for the unitary current and using the autocorrelation of the macrocurrent while neglecting information from averaged unitary currents (see METHODS). The time course of P(t) and the waveform of the unitary current, as deduced from this approach, were reasonably consistent with the corresponding estimates using the averaged unitary currents (Fig. 7B). However the decay time constants obtained with this approach were systematically longer than those fit to the averaged microcurrents (Table 1).

This discrepancy might be the result of an intrinsic error in the procedure or, alternatively, of a flaw in the original assumption of P(t) being faster than the unitary event. In order to investigate more deeply this point, the time integrals of P(t), as estimated with the two procedures in the same neuron, were compared. An example is displayed in Fig. 7, A and B. The time integral of P(t), F(t), monitors the cumulative number of unitary currents (i.e., the expected total number of quanta of ACh that were released) at any time, after the delivery of the stimulus. Data in Fig. 7 reveal that, if the macrocurrent is generated by unitary currents with the same waveform as those occurring spontaneously, the probability of release displays an early pulsing behavior (see also Fig. 1), giving rise to a step in the integral of P(t), followed by a late, small, slowly decaying tail indicated by the persisting positive slope in the integral, F(t). This small tail accounts for ~15% of total release, which occurs over a period of ~20 ms after the synchronous pulse and is apparent and qualitatively quite similar with both approaches (compare Fig. 7, A and B). The tail is slightly underestimated by the autocorrelation approach: this explains the discrepancy in time-constant estimates and highlights the fact that the assumption of P(t) being faster than the unitary event is almost true, but not quite so, its tail presumably reflecting facilitation of release during a short time period after stimulation.

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<tr>
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EPSC, excitatory postsynaptic current.
QUANTUM CONTENT OF EVOKED SYNAPTIC CURRENTS. The quantum content of averaged macrocurrents was estimated as the ratio of the area under the mean unitary event, recorded from the same cell, to the area under the EPSC. The mean quantal content ranged between 30.2 and 88.7, with a mean value of 56.1 in five different neurons, during long-lasting trains at 10 Hz. A higher estimate of quantum content, some 120 quanta per stimulus, is obtained by comparing peak macrocurrent conductance in the experiment illustrated in Fig. 3A to the estimated peak microcurrent conductance. However, in the latter case synaptic currents were evoked in isolation once every 20 s, a condition where the quantum content is physiologically expected to be higher.

Interactions between the synaptic current and the postsynaptic conductances

A MODEL FOR THE EPSC. We have devoted particular attention to the analysis of macrocurrent onset, when evoked at different membrane potential levels; it always proved to be well described by a single exponential function. The numerical values of the associated time constant, $\tau_\text{t}$, were obtained by solving the equation (Bonifazzi et al. 1988)

$$t_0 = \frac{\tau_1 \tau_2}{\tau_2 - \tau_1} \ln \left( \frac{\tau_1}{\tau_2} \right)$$

(1)

where $t_0$ is the EPSC time-to-peak and $\tau_1$ the current decay time constant, extracted directly from the current tracings. The rise time constant proved to be virtually voltage-independent over a wide voltage range and had a mean value of 0.57 ms in fourteen neurons. This would indicate that at ganglion cells ACh binding and channel opening rate constants have little or no voltage sensitivity, a conclusion consistent with the observations at other cholinergically gated receptor channel complexes (Auerbach et al. 1996; Connor et al. 1983; Dionne 1981; Rang 1981). We decided, therefore, to describe the synaptic macrocurrent by the following double exponential equation

$$I = g_0 [V(t) - E_r] \cdot \left[ \exp \left( -\frac{t}{\tau_1} \right) - \exp \left( -\frac{t}{\tau_2} \right) \right]$$

(2)

where $g_0$ is the synaptic conductance and $E_r$ the synaptic current reversal potential. This equation not only represents the best phenomenological description of the EPSC time course (better fits were obtained than by using gamma or alpha functions) but is also predicted by the conventional three-state kinetic scheme: $nA + R \rightleftharpoons A_1R \rightleftharpoons A_2R \rightleftharpoons A_3R^*$. This scheme predicts a synaptic current that decays as a single exponential function of time, $\tau_1$, whereas the additional time constant, $\tau_2$, accounts for the rise phase and the time-to-peak of the EPSC. The results presented so far suggest that, in spite of the great difference between the amplitude of the EPSCs and mEPSCs, their time courses are similar; the time constant of either micro- or macrocurrent onset is essentially unchanged even when the peak EPSC amplitude is 100-fold that of a mEPSC. Similarly, the indicated mean mEPSC decay time constant (4.51 ms at $-75$/$-120$ mV) favors comparisons with the $\tau_1$ values for EPSCs in Fig. 3B. The overall response is thus well described by the sum of a large number of small mEPSCs. Although some temporal dispersion in time of release of individual quanta occurs, release probability as a function of time can be compressed, under appropriate conditions, to a very sharp Gaussian distribution (Fig. 7, A and B).

The lines through $\tau_1$ points in Figs. 3, A and B, indicate the momentary value of the decay time constant at any potential, in either 5 or 2 mM external Ca$^{2+}$, respectively. The only remaining parameter to estimate in Eq. 2 is $g_0$, which is obviously related, among others, to the extent of presynaptic input recruitment, the external Ca$^{2+}$ concentration, the frequency of synaptic stimulation. A mean $g_0 = 0.58 \mu S$ per neuron (where $g_0$ indicates the cumulative peak conductance when all the synaptic channels activated during the EPSC are simultaneously open) was estimated in 5 mM Ca$^{2+}$ solution. The data in Fig. 3 provide the appropriate correction for $g_0$, in physiological, 2 mM external Ca$^{2+}$ solution. The time course of the synaptic currents simulated by using Eq. 2 with these parameters agreed well with experimental results. The calculations, in fact, reproduced the observed growth phase and the exponential decay. Similarly, computed and measured time-to-peak estimates consistently indicated a mean value of 1.9 ms. The numerical values and equations used to compute the fast nicotinic synaptic currents for our standard cell, in either 5 mM or 2 mM external Ca$^{2+}$ solution, are summarized in Table 2, for clarity.

**Table 2.** Mean values of the constants and voltage dependence of $\tau_1$ used to reconstruct the ganglionic EPSC in the presence of different Ca$^{2+}$ external concentrations

<table>
<thead>
<tr>
<th>Ca$^{2+}$ concentration</th>
<th>$g_0$ ($\mu S$)</th>
<th>$\tau_1$ (ms)</th>
<th>$\tau_2$ (ms)</th>
<th>$E_r$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 mM Ca$^{2+}$</td>
<td>0.40 $\mu S$</td>
<td>0.57 ms</td>
<td>4.34 $\exp(-0.00413 \cdot V)$</td>
<td>$-15.7$ mV</td>
</tr>
<tr>
<td>5 mM Ca$^{2+}$</td>
<td>0.58 $\mu S$</td>
<td>0.57 ms</td>
<td>5.56 $\exp(-0.00384 \cdot V)$</td>
<td>$-15.7$ mV</td>
</tr>
</tbody>
</table>

$\tau_1$, coefficient of voltage dependence is expressed in mV$^{-1}$; $\tau_1$ in ms. EPSC, excitatory postsynaptic current.

MODEL FOR $I_A$, THE MAIN VOLTAGE DEPENDENT CURRENT IN THE SUBTHRESHOLD RANGE. The $I_A$ in the sympathetic neuron was previously characterized in voltage-clamp experiments (Belluzzi et al. 1985a). It develops above a threshold voltage of $-60$ mV and obeys third-order kinetics with a fast activation time constant (0.3 ms at $-10$ mV). The steady-state value of the activation variable $a$ exhibits a strong voltage dependence (14.4 mV are required to change $a_s$ by e-fold) and it is half-activated at $-58.5$ mV. The $I_A$ channels provide the neuron with a large conductance that increases with depolarization up to a maximum of 2.3 $\mu S$ per cell at 10 mV. The $I_A$ current, on the other hand, is completely inactivated at $-50$ mV; the steady-state inactivation curve covers the range from $-100$ to $-50$ mV, with a midpoint at $-78$ mV, suggesting that a large fraction of $I_A$ channels are potentially available at the physiological resting potential. This current is the only ionic current component activatable in the spike subthreshold range, as indicated by the position of the activation curve (partially illustrated in Fig. 8A, inset).
The effects of $I_{A}$ activation during voltage shifts produced by current steps, injected through the current electrode, were systematically investigated under two-electrode constant current conditions and reproduced with the mathematical model of the neuron. This analysis was aimed at further validating the model and clarifying the interplay between holding potential value, which controls the fraction of the activatable $A$ channels, and membrane potential peak depolarization, which determines the number of $I_{A}$ channels actually opened by the voltage shift.

Figure 8A shows a family of electrotonic potentials elicited in a neuron by 1-ms current pulses either in hyperpolarizing or in depolarizing direction. The holding potential was about $-90$ mV, which corresponds to a steady-state $I_{A}$ inactivation fraction higher than 0.8. The electrotonic potentials reaching short of the $I_{A}$ activation voltage all decayed with the same exponential time course, independent of polarity ($traces$ $a$ -- $c$ and $e$), suggesting that only the passive electrical properties of the neuron were being involved by current flow. Distortions of the signal from the pure electrotonic potential were observed when the membrane potential moved into the region of $I_{A}$ activation ($trace$ $d$); the decay of the signals observed under these conditions can be reasonably well split into the sum of two exponentials, a faster one ($0.67$ ms time constant), which drives the signal decay just after the peak depolarization and a slower one which corresponds to the major cell time constant ($3.57$ ms) detected in the previous analyses. A similar behavior is illustrated in a different neuron (Fig. 8D), in which wider membrane shifts were imposed, starting from a constant $-99$ mV holding potential. The membrane deflection displayed a biphasic decline when the $I_{A}$ activation range was reached: the early part of the decay was much faster than the late part, which was eventually governed by the major cell input time constant ($0.46$ ms; time constants $3.63$ ms), exhibiting an early, fast decay time constant, which becomes progressively shorter as the size of the voltage steps is increased within the $I_{A}$ activation voltage range, whereas the main (slower) cell time constant remains unchanged and is readily extracted in all tracings from the final part of the response.

The time course of the membrane voltage shifts after current step injection is accurately mimicked by the computer model of the sympathetic neuron incorporating the kinetic properties of each current type. In Fig. 8B, the active currents presumably involved in generating the signals of Fig. 8A are represented. These currents generate the computed curves in Fig. 8C. The model accurately reproduces the shape of the electrotonic potentials and the experimentally observed effects; in the $I_{A}$ activation range, in fact, the departures from the passive cell behavior are identical to recorded
tracings. The inward current steps that sustain the largest depolarizations are unequivocally contaminated by a substantial $I_A$ outward flow (see Fig. 8Bd), which alone generates the distortion in the early signal decay. As observed experimentally, when the neuron holding potential was moved into the voltage range of $I_A$ steady-state inactivation, the decay of the signals after current injection was predicted by the passive membrane behavior alone; conversely, as the holding potential was made progressively more negative, steps to potentials where the activation curve indicated the presence of $I_A$ elicited an increasingly relevant contribution by the faster time constant after the peak depolarization.

INTERACTIONS BETWEEN EPSC AND $I_A$ IN THE SUBTHRESHOLD RANGE. The analysis in the previous section confirms the important contribution of $I_A$ in the subthreshold potential range. A similar, systematic investigation of the interactions between the synaptic current and the active neuronal conductances (and $I_A$ in particular) would require producing synaptic potentials of graded amplitude. This turned out to be a quite demanding task, because of the intrinsic nonlinearity in the recruitment of the preganglionic input onto the neuron; however, these data could be combined with voltage step results and the predictions of the mathematical model of the neuron to yield a comprehensive picture of the current flows during the synaptic response.

Evidence for a prompt activation of $A$ channels during the EPSP time course was obtained experimentally in response to electrical stimulation of the preganglionic nerve. In Fig. 9A, the presynaptic stimulus intensity was adjusted so as to obtain a subthreshold depolarization of the postsynaptic neuron, which was held at different membrane potentials negative to $-80$ mV by a constant current flow. When the peak of the EPSP was sufficiently large to reach the $I_A$ activation voltage range (Fig. 9A, inset), a fast membrane repolarizing phase became evident at the peak of the synaptic potential—similar in nature and position to the fast time constant component detected in response to current pulses—and was followed by a prominent hump in the EPSP shape. When the neuron was artificially hyperpolarized by injecting current through the current electrode, the new EPSP peak reached lower levels and the fast falling phase became less evident (Fig. 9Ab). The EPSP eventually reverted to the usual time course when it became unable to activate the $I_A$ current to a significant extent (Fig. 9Ac). In the neuron shown in Fig. 9D, a presynaptic stimulation of constant magnitude was able to fire the cell from an initial potential of $-82$ mV, but it became subthreshold when the membrane potential was shifted to $-93$ mV. This experiment unequivocally demonstrates the common nature of the ionic mechanisms that underlie the fast spike falling phase as well as the EPSP distortion around its peak.

The shapes of the EPSPs generated by our mathematical model were compared with the experimental results and analyzed to further substantiate these conclusions. Synaptic conductances about $0.3 \, \mu S$ in peak amplitude generated computed synaptic potentials (Fig. 9B), which accurately mimicked the records illustrated in Fig. 9A at the appropriate membrane potential. The underlying currents shown in Fig. 9C clearly illustrate the interplay of synaptic and $I_A$ currents in generating the EPSP of Fig. 9Ba and the almost pure synaptic nature of that in Fig. 9Bc, where the EPSP amplitude is of inadequate magnitude to generate any detectable $I_A$. The time courses of the synaptic and $I_A$ macrocurrents can be observed in isolation in Fig. 9Ca. It will be noted that $I_A$ activation is fast and the current displays deactivation kinetics slightly faster than the decay of the synaptic current (and increasingly fast as the membrane potential is made more negative, as opposed to the negligible voltage sensitivity of $\tau_{I_A}$). Thus the effects of $I_A$ flow are precocious and clearly noticeable, although transient; the balance between synaptic and $K^+$ currents results in a persistently inward net current, which sustains a long-lasting membrane depolarization (see Fig. 9D).
I_A DURING THE SYNAPTICALLY EVOKED SPIKE. In the previous section we have demonstrated that the synaptic potentials can be sufficiently fast to avoid voltage-dependent steady-state I_A inactivation and sufficiently large to activate a significant amount of A channels during the subthreshold voltage trajectory. Thus under appropriate circumstances the transient outward current, initiated by EPSPs moving in and out of the range where I_A is activated, exerts a short-lived hyperpolarizing influence, which is expected to reduce neuronal excitability. Intuitively, this was suggested in earlier studies (Cassell and McLachlan 1986; Daut 1973; Segal et al. 1984). The actual effect of the interaction between synaptic and I_A currents in terms of excitability control of neuronal firing is studied quantitatively here and analyzed by means of a reliable model of the sympathetic neuron soma based on the characterization of the individual current components.

The amount of charge required for square 3-ms current steps to bring the current-clamped sympathetic neuron to the action-potential firing threshold is plotted in Fig. 10A against the holding potential, in the −40/−120 mV voltage range. The threshold charge during the depolarizing pulse displays a nonlinear dependence on the starting membrane potential. The figure also illustrates that the change in threshold, with increasing negativity, parallels the voltage dependence of I_A steady-state inactivation removal; in particular, anomalous rectification is not found in these cells and the passive membrane behavior is apparently linear at potentials negative to −40 mV. The same sigmoidal relationship between threshold charge and initial holding potential is obtained when similar current injections are applied to the artificial model of the neuron (Fig. 10B, ▽) whereas the relation becomes linear if the I_A is neglected in the simulations (Fig. 10B, ▼). The comparison of the curves of Fig. 10B gives a direct measure of the I_A participation in damping neuronal excitability. In the voltage range of physiological inactivation of I_A no significant differences are produced by omitting I_A, as expected. The I_A role becomes increasingly evident when membrane potential removes I_A inactivation; at −80 mV, for example, the I_A contribution increases the amount of outward charge needed to trigger a single spike by a factor of 1.84. A similar behavior was revealed in the simulations when the neuron was activated by the natural synaptic input (Fig. 10C), as described in the previous sections. This was the only feasible approach to thoroughly test this point. The neuronal excitability machinery is now moved by the physiological mechanisms and the results provide a quantitative confirmation of the previous conclusion, namely that the transient outward current reduces the excitability of neurons at holding potentials negative enough for I_A inactivation to be removed. The voltage dependence of this effect is again well-predicted by the I_A steady-state inactivation curve. An inhibitory effect on excitability is now detected in the presence of the I_A current component and the magnitude of such inhibition is comparable with that observed with current pulses: the synaptic peak conductance necessary to fire the neuron held at −80 mV increases by a 1.87 factor when I_A is included in the model.

IONIC CURRENTS UNDERLYING THE SYNAPTICALLY EVOKED SPIKE. The detailed time course of the different ionic currents underlying a synaptically evoked spike is illustrated in Fig. 11. The action potential is fired by a reconstructed synaptic input applied to the neuron from different holding potentials (−50 vs. −75 mV) to specify to what extent I_A contributes to the firing pattern. As concerns the synaptic current under current-clamp conditions (Fig. 11B) it will be noted that it reverses according to the voltage excursions across its equilibrium potential and that its long-lasting inward late component (the voltage does not help the synaptic current to turn off) engenders the delayed depolarization particularly evident in the −75 mV spike. This effect is observed in the experimental action potential (see Fig. 9D) and is not related to asynchronous neurotransmitter release but simply to the closing kinetics of the synaptic channels underlying the strong compound EPSP. The presence of a strong rectification blocking any outward current flow
The dominant role of $I_A$ as repolarizing current in the $-75$ mV spike (Fig. 11C) is expected from previous analyses (Belluzzi et al. 1985a; Belluzzi and Sacchi 1991). Moreover, comparison of the shape of this spike to that evoked by direct stimulation indicates that the peak falling rate is unaffected by the concomitant presence of a synaptic conductance. A modest reduction in peak repolarization rate is observed only at more negative holding potentials (some 12% at $-90$ mV) and is most likely related to the shunting action of the large synaptic current.

Changes in the voltage threshold for action-potential generation (at about $-45$ mV in the ideal neuron) because of the presence or absence of $I_A$, as suggested by Segal et al. (1984), were not detected in the present experiments. $I_A$, on the contrary, mainly contributes to shaping the total transmembrane active current at the crucial moment, which anticipates the massive $I_{Na}$ invasion. This is clearly shown in Fig. 11D. The synaptic current component in the $-50$ mV spike, in the absence of substantial contribution from the largely inactivated $I_A$, smoothly continues into the $I_{Na}$ inward component. During the $-75$ mV spike instead, as $I_A$ has a significantly lower threshold for activation than the $Na^+$ current, the outward current onset actively shunts the synaptic current, until $I_{Na}$ undergoes sufficient voltage-dependent activation to make the total inward current prevail and drive the cell to the spike. The practical result is to temporally split the synaptic excitation from the large $I_{Na}$ inflow, thereby delaying the full development of the action potential.

**DISCUSSION**

Almost pure nicotinic responses were recorded after presynaptic stimulation and characterized, both at the macrocurrent level and at the single quantum level, in the rat sympathetic neuron. The compound EPSC was reconstructed on the hypothesis of a quasi synchronous presynaptic release of quanta from different release sites distributed on soma and dendrites of an isopotential cell. We have then combined the experimental data about the synaptic activity with previous information about the excitability properties of the neuron to examine the interaction between synaptic drive and action potential generation.

The waveform can be estimated with reasonable accuracy by deducing their time constants from the autocorrelation of the macrocurrents. Second, on the basis of the assumption that the time course of the unitary current remains unchanged during the EPSC, the deconvolution procedures provide estimated time courses of the probability of ACh release after preganglionic nerve stimulation, even when the time constants of the unitary events are unknown. When the unitary events can be analyzed, the time course of release probability can be more accurately evaluated to obtain information not
only on the phasic release of ACh after stimulation, but also on the early time course of subsequent facilitation of release. Although alternative interpretations can be proposed for the observed tail in evoked release (e.g., changes in the unitary event time course), these analyses make it possible to simulate the synaptic input onto the neuron even when release becomes asynchronous, as it occurs during the natural ongoing preganglionic activity.

The most significant outcome of this analysis is the demonstration that $I_A$ potentially exerts a second relevant function in the neuronal firing strategy, in addition to the well-defined role of providing the main recharging current during the action-potential falling phase (Belluzzi et al. 1985a), namely a direct control of the cell excitability preceding its suprathreshold full activation. The $A$ channel begins activating much earlier, during the rising phase of the action potential, than does any other ionic channel, and the amount of time during which the membrane potential is above threshold is therefore longer for $A$ channels than for other channels. The $I_A$ seems to reduce the excitability of neurons at holding (or resting) potentials negative enough for $I_N$ inactivation removal. This effect is strongly voltage dependent and mirrors the steady-state $I_N$ inactivation curve. If $I_A$ reduces neuronal excitability, its block is expected to decrease not only the repolarizing power during the spike development, but also the amount of current necessary to discharge the membrane capacitor to the firing threshold. One of the most common antiepileptic drugs, carbamazepine, was recently reported to enhance the $A$-conductance at therapeutic concentrations (Zona and Avoli 1990) and, conversely, the unspecific $I_A$ blocker, 4-aminopyridine, is a well-known seizure promoter.

The observation that the initial membrane potential level is a crucial parameter in the cell firing strategy is recurrent in our analyses. In earlier studies we showed that membrane potential controls the mix of different channel types available on the cell to generate different firing patterns (Belluzzi and Sacchi 1991). In the present study we demonstrate that the spike discharge itself is controlled by the underlying $I_A$, which coexists with the synaptic current flow. In the absence of any definite knowledge about the mechanisms governing the actual membrane potential level of a neuron, it may be assumed that the spike discharge intensity might represent one of the relevant controllers of the neuron membrane potential, via the $Na^+$ load and the resulting electrogenic $Na^+$ extrusion. It is tempting to correlate the postsynaptic mechanism here described, which regulates the level of synaptic stimulation needed to fire an action potential, with the extensively studied pre-synaptic modulatory mechanisms such as potentiation, augmentation, facilitation, and long-term potentiation. These processes are known to operate also at the ganglionic synapse (Briggs and McAfee 1988; Brown and McAfee 1982; Koyano et al. 1985; Zengel et al. 1980, reviewed by Briggs 1995) and to increase synaptic efficiency based on the same triggering event, namely intense firing activity; this might reflect an adaptive process to achieve a constant safety factor for transmission through the synapse.

The present experiments, together with already available morphological and electrophysiological data under current-clamp conditions in the same preparation, provide a comprehensive picture of the ganglionic synapse microphysiology. Each rat sympathetic neuron receives synaptic contacts from 6–9 preganglionic fibers (Purves et al. 1986; Sacchi and Perri 1973), which supply the cell with a mean of 330 somadendritic synapses (Forehand 1985). The present analyses, in which the compound synaptic macro- and microcurrent amplitudes were compared, have demonstrated that the quantum content of EPSCs at the beginning of a 10-Hz train of supramaximal stimuli applied to the sympathetic trunk is 56.1 in 5 mM Ca$^{2+}$ external solution, which yields a mean value of 38 quanta per stimulus after correction for the normal 2 mM Ca$^{2+}$ external complement. This would correspond to a quantum content of 4.2–6.3 per single preganglionic fiber. This figure favorably compares with an average quantum content of $4.5 (n = 12)$ independently evaluated in the rat ganglion from statistical analysis of EPSP fluctuations during the first minute of 10 Hz stimulation of a single preganglionic fiber (Sacchi and Perri 1973). Assuming that the mean conductance of the nicotinic ACh channels in dissociated or cultured rat sympathetic neuron is 35 pS (in the presence of 1 mM Ca$^{2+}$) (Mathie et al. 1987, 1991) or 20 pS (in 2.5 mM Ca$^{2+}$) (Derkach et al. 1987), a single quantum of ACh would appear to open 115–200 synaptic channels at the peak of the mEPSC. This is much less than the 2,000 nicotinic channels opened by a single quantum of ACh at the frog neuromuscular junction (Anderson and Stevens 1973), but is consistent with existing estimates for other nicotinic receptor channels in mammalian peripheral neurons (Derkach et al. 1983; Rang 1981). Similarly, the amplitude ratio of the largest measured synaptic conductance to single channel conductance would suggest that some 30,000 functional nicotinic receptor channels per neuron are activated by each supramaximal preganglionic stimulus. The relatively low quantum content value indicates that no more than 11% of the synaptic boutons is activated by the same stimulus, assuming that the single contact releases at most one quantum after an action potential. This would intuitively suggest that the cumulative probability of quantal emission at the ganglionic synapse is low, as indicated by previous statistical analyses on synchronous and K$^+$-evoked quantal release (Sacchi and Perri 1971, 1973, 1976). Binomial analyses of quantal contents of EPSCs during the 10 Hz trains yielded very low values for $P$ in some portions of the trains (suggesting that release actually obeyed Poisson statistics), whereas in other portions $P$ was even $>0.5$ and Poisson analysis of quantal content gave markedly inconsistent results. All this points to markedly inhomogeneous probability of release from the various boutons and to variations in probability of release with time, which prevent an accurate evaluation of the statistics of quantal release in the absence of quantitative assessment of the spatial and temporal variance in the probability of quantal release from the same unit (Barton and Cohen 1977; Brown et al. 1976; Perkel and Feldman 1979).

Earlier data under current-clamp conditions indicated that the simultaneous release of some 8–10 quanta was necessary to drive the rat ganglion neuron to its firing threshold (as derived from a mean quantum size as high as 1.8 mV) and that the safety factor for synaptic transmission appeared to be about four, when the whole presynaptic input was activated...
(Sacchi and Perri 1973). These estimates are appropriate, according to the present evidence, only for a neuron exhibiting a membrane potential in the range of −50 or −60 mV. More negative membrane potentials will necessitate that a larger number of quanta be secreted to induce an action potential not only because of their nonlinear summation, but also to face the additional major source of nonlinearity arising from the concomitant $I_N$ flow. The initially high safety factor for synaptic transmission would suggest that a low convergence degree, of one or a few active preganglionic fibers, is sufficient to evoke a spike; when the safety factor is progressively reduced by hyperpolarization a complete concentration of the convergent innervation is required to fire the cell. This raises the question as to the functional effects of ongoing activity in the sympathetic preganglionic fibers. Discharge evoked in sympathetic postganglionic neurons may be generated by the activation of single ‘‘strong’’ preganglionic fibers capable of evoking large EPSPs or by groups of more or less synchronously discharging ‘‘weak’’ preganglionic fibers (recently reviewed by Jänig 1995). It is unclear which type of mechanism operates in the different neurons and how the synchronous firing of preganglionic fibers is generated. Whatever the organization of the preganglionic input and the ways impulses are transmitted in vivo, the present findings clearly indicate that the success in initiating the postganglionic firing will critically depend on the momentary level of the sympathetic neuron resting potential.

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