Nicotinic EPSCs in Intact Rat Ganglia Feature Depression Except If Evoked During Intermittent Postsynaptic Depolarization

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Nicotinic EPSCs in intact rat ganglia feature depression except if evoked during intermittent postsynaptic depolarization. J Neurophysiol 83: 3254–3263, 2000. The involvement of the postsynaptic membrane potential level in controlling synaptic strength at the ganglionic synapse was studied by recording nicotinic fast synaptic currents (EPSCs) from neurons in the intact, mature rat superior cervical ganglion, using the two-electrode voltage-clamp technique. EPSCs were evoked by 0.05-Hz supramaximal stimulation of the preganglionic sympathetic trunk over long periods; their peak amplitude (or synaptic charge transfer) over time appeared to depend on the potential level of the neuronal membrane where the nicotinic receptors are embedded. EPSC amplitude remained constant (n = 6) only if ACh was released within repeated depolarizing steps of the postganglionic neuron, which constantly varied between -50 and -20 mV in consecutive 10-mV steps, whereas it decreased progressively by 45% (n = 9) within 14 min when the sympathetic neuron was held at constant membrane potential. Synaptic channel activation, channel ionic permeation and depolarization of the membrane in which the nicotinic receptor is localized must occur simultaneously to maintain constant synaptic strength at the ganglionic synapse during low-rate stimulation (0.03–1 Hz). Different posttetanic (20 Hz for 10 s) behaviors were observed depending on the mode of previous stimulation. In the neuron maintained at constant holding potential during low-rate stimulation, the depressed EPSC showed posttetanic potentiation, recovering ~23% of the mean pretetanic values (n = 10). The maximum effect was immediate in 40% of the neurons tested and developed over a 3- to 6-min period in the others; thereafter potentiation vanished within 40 min of 0.05-Hz stimulation. In contrast, no statistically significant synaptic potentiation was observed when EPSC amplitudes were kept constant by repeated ~50/-20-mV command cycles (n = 12). It is suggested that, under these conditions, posttetanic potentiation could represent an attempt at recovering the synaptic strength lost during inappropriate functioning of the ganglionic synapse.

INTRODUCTION

Sympathetic ganglia have frequently been used as models for the study of neuronal synaptic transmission and of the processes modulating synaptic function. Mammalian ganglia possess a multitude of neuroregulatory mechanisms; some of them affect or are affected by preganglionic cholinergic activity. Ganglionic posttetanic potentiation is one of the first use-dependent synaptic poteniations that have been observed, and neurogenic long-term potentiation represents a well-known posttetany effect even in the cholinergic sympathetic pathway (for a review see Briggs 1995). Apart from problems in terminology, a relatively brief conditioning preganglionic stimulation results in modifications of synaptic function over periods ranging from milliseconds to hours. These effects have been studied electrophysiologically by using extracellular mass recordings from postganglionic trunks, or intracellular recordings under current-clamp conditions. The parameters conventionally utilized to measure the relative efficacy of synaptic transmission were therefore the amplitude of either the postganglionic compound action potential or the excitatory postsynaptic potentials (EPSPs) recorded intracellularly in a single neuron. Analysis of miniature EPSP (mEPSP) amplitude distribution represented a complementary tool applied in an attempt to separate the pre- and postsynaptic components (Koyano et al. 1985). Neurogenic nicotinic long-term potentiation (LTP) has been observed to last for several hours in vitro in rat superior cervical ganglia (SCG) (Briggs et al. 1985; Brown and McAfee 1982) and frog sympathetic ganglia (Koyano et al. 1985; Kuba and Kumamoto 1990); in rat ganglia a brief tetanic preganglionic stimulation resulted in a two- to threefold potentiation of the fast EPSP lasting up to 3 h (Briggs and McAfee 1988), and a similar strong potentiation was observed in frog ganglia.

In all these experiments a detailed phenomenological description was not accompanied by a parallel understanding of the basic aspects underlying potentiation, especially as concerns the biophysical properties of the postsynaptic receptors; most observations were actually performed under current-clamp conditions, and the postsynaptic properties were tested by applying exogenous neurotransmitter. In the present experiments the nicotinic synaptic transmission was studied in intact rat ganglia, before and after preganglionic tetanization, by using the two-microelectrode voltage-clamp technique. Large synaptic currents are thus recorded while precisely controlling the membrane potential of the postganglionic neuron under conditions in which ACh is released from naturally developed nerve terminals (Sacchi et al. 1998). Control of the postsynaptic membrane potential appeared to be an additional, and unexpected, factor of crucial importance in maintaining synaptic transmission efficacy, which could only be kept constant, even at very low rate of ACh release, by preserving precise timing between ACh exposure and potential modification of the membrane hosting the ACh receptor. The application of ACh onto a recurrently depolarized neuron, and exclusively during the depolarization, appeared to be a prerequisite for maintaining a synaptic current of constant amplitude at a low preganglionic stimulation rate; if these processes were dissociated, a progressive deterioration of synaptic efficacy ensued. This depression is determinant in synapse posttetanic behavior.
METHODS

Experiments were performed on superior cervical ganglia isolated from rats (male or female; 130–150 g body wt) during urethane anesthesia (1.5 g kg⁻¹ ip) and maintained in vitro at 37°C. In some experiments the ganglion was dissected from the animal 2 h after the initial anesthesia and sectioning of the sympathetic trunk; in others the isolated ganglia were maintained for 2 h unstimulated in vitro before starting with the electrophysiological experiment; the latter conditions did not appear to affect subsequent behavior. After surgery, the animals were killed with an overdose of anesthetic. Ganglia, including the preganglionic sympathetic trunk and postganglionic neurons, were mounted on the stage of an upright microscope (Zeiss UEM); superficial neurons were identified at a magnification of ×500 by using diffraction interference optics and impaled with two independent glass microelectrodes filled with neutralized 4 M K⁺ acetate and having resistances of 30–40 MΩ. In a few experiments 1,2-bis(2-aminophenoyx)ethane-N,N',N''-tetraacetic acid (tetrapotassium salt, BAPTA; Sigma) was dissolved in the microelectrode filling solution to a final concentration of 170 mM. Recordings were obtained under two-electrode voltage-clamp conditions as previously described (Belluzzi et al. 1985). Ganglia were continuously superfused with a medium pregassed with 95% O₂:5% CO₂ to a final pH 7.3 and had the following ionic composition (in mM): 136 NaCl, 5.6 KCl, 5 CaCl₂, 1.2 MgCl₂, 1.2 NaH₂PO₄, 14.3 NaHCO₃, and 5.5 glucose. Choline chloride 10⁻³ M and atropine sulfate 1–2 × 10⁻⁹ M were added to the saline, unless otherwise stated. In some cases the normal solution was diluted 1:1 with a synthetic culture medium (Dulbecco’s modified Eagle medium) keeping the calcium concentration constant at 5 mM. The bath was grounded through an agar-3 M KCl bridge.

To activate the preganglionic input, supramaximal single current pulses of 0.3 ms duration were applied at 0.05 Hz to the cervical sympathetic trunk through a fine suction electrode, either while the neuron was kept at a constant holding potential (usually −50 mV) or during the application of repetitive cycles where the postsynaptic membrane potential was commanded to different voltages. Under the latter conditions, the usual protocol was to jump every 20 s to a different test potential in the −20/−50-mV range, in 10-mV steps, 40 ms before the preganglionic shock was delivered and returning to the holding level 60 ms after the stimulus. Each cycle started with the −20-mV command pulse and was continuously repeated, so that the EPSC was observed at the same membrane potential level only once every 80 s. Great attention was taken during the preliminary procedures to avoid any stimulation of the presynaptic fibers; only direct stimulation of the neuron through the current electrode was used to recognize the cell and evaluate the quality of the recording, whereas preganglionic stimulation started exclusively with the onset of the experiment. Preganglionic tetanization was represented by an isolated 10-s train of 20-Hz supramaximal stimuli while the neuron was clamped at −50 mV. The ACh equilibrium potential (EACₐ) was evaluated at the end of the experiment by extrapolating to zero current the voltage-current (I-V) excitatory postsynaptic current (EPSC) peak amplitude relationship obtained over the −80/−30-mV voltage range (Sacchi et al. 1998), or by occasionally adding the −80/−60-mV sequence to the normal 0.05-Hz −50/−20-mV cycles during the course of the experiment.

Large synaptic currents were recorded with good control of the membrane potential at any tested voltage; single currents were filtered at 5 kHz with an 8-pole Bessel filter, digitized at 10 kHz with a 12-bit A/D interface (Digidata 1200A operating pCLAMP software, Axon Instruments) and stored on disk for further analysis. Long-lasting current tracings were filtered at 5 kHz and digitized continuously on tape (Biologic, DTR-1200; 0–10 kHz). Data were analyzed on Pentium personal computers (AST) with pCLAMP (Axon Instruments) software package.

Statistical analysis was performed on absolute or normalized values (with respect to the 1st EPSC of each series) by using unpaired t-tests when comparing isochronal data points under two different experimental conditions. The difference between the time course over time of EPSC peak values was assessed by comparing (t-test) the regression coefficients (b) across the two series of time points when two different voltage-clamp protocols were applied. The level of significance is indicated in the text.

RESULTS

General EPSC properties at low stimulation rate and after tetanization

Figure 1 shows the results obtained from a rat sympathetic neuron, in which the nicotinic EPSC was recorded for an unusually long-lasting period, making it possible to test a mix of different preganglionic stimulation rates and postganglionic membrane potential modifications in the same cell. The supramaximal preganglionic stimulation at 0.05 Hz started with the neuron constantly held at −50 mV, in a bathing medium enriched with a tissue culture medium. The EPSC peak amplitude was not maintained, but decreased by ≈43% of its maximum value within 15 min. A 20-Hz tetanus of 10 s duration (1st arrow) only partially and transiently compensated for this initial decay: the EPSC amplitude tested systematically at −50 mV every 20 s slowly increased but thereafter returned to the pretetanus values within 45 min. A different protocol was then applied to the neuron, so as to observe the EPSC at different membrane potential levels over the −50/−20-mV range (and later over the −70/−20-mV range), whereas keeping the 0.05-Hz preganglionic stimulation rate constant. Cutting off perfusion with the enriched medium and returning to normal saline solution (*) did not apparently modify the synaptic efficacy. A second tetanus (2nd arrow) was applied two h after onset of recording with minor effect on EPSC amplitude, compared with that observed after the first tetanus. The experiment was concluded with a final period at a constant −50-mV holding potential. The 0.05-Hz stimulation rate was maintained throughout; during this period neither the EPSC decay time constant at the different membrane potentials tested nor the ACh equilibrium potential were significantly modified. This general behavior was also confirmed when, instead of EPSC peak amplitude, synaptic charge displacement was taken into account. Three different factors are apparently involved in controlling synaptic efficacy during constant rate preganglionic stimulation, namely 1) the level of the neuron holding potential, 2) repeated voltage modifications of the postsynaptic membrane potential, and 3) application of a preganglionic tetanus. Each of these aspects is analyzed separately.

EPSC peak amplitude during low rate preganglionic stimulation

The major effect observed in the previous experiment is possibly the initial fade in EPSC amplitude during 0.05-Hz stimulation, whereas the neuron membrane potential was kept constant. This observation was confirmed in a group of nine neurons maintained at a stable holding potential of −50 mV, in which the mean EPSC peak amplitude progressively declined by 45% during a 14-min period of 0.05-Hz preganglionic stimulation, with an apparent exponential time course (τ = 6–8 min; Fig. 2, A and D, ●). This result cannot be readily explained by modifications in the ACh volley output from the
presynaptic terminals, because it remained virtually constant in neurochemical experiments on the isolated rat SCG, even during a 60-min stimulation period at 10 Hz (Sacchi et al. 1978).

Quite a different picture arose when the “presynaptic” part of the experiment was left unchanged but the EPSC was evoked at variable membrane potential levels of the postsynaptic neuron; the holding potential was kept at 250 mV while the EPSC was now evoked during voltage commands of 100 ms duration, and varying in 10-mV steps within the range 220/250 mV, so as to measure the EPSC at 250 mV once every 80 s. Under these conditions the EPSC amplitudes at the different membrane potentials were virtually unchanged over the 14-min low-frequency stimulation that produced a clear-cut EPSC rundown in the absence of postsynaptic membrane potential migrations. A typical experiment is partially illustrated in Fig. 2B and C, whereas in Fig. 2D the open circles show the 250-mV mean values (n = 6). Point-by-point comparisons (unpaired t-test) indicate a significant difference (P < 0.05) after 14 min of stimulation when absolute data are considered; the same level of significance is obtained after 3-min stimulation (with P becoming <0.001 after 4 min) if normalized data are used. The regression coefficient (b) was not significantly different from zero when the protocol with recurrent 250/220-mV cycles was applied, but became highly significant (P < 0.001) when the holding potential was kept constant. Comparison between the two b values indicates that the time course of EPSC amplitude over time is significantly different (P < 0.001) during the application of the two voltage-clamp protocols used in Fig. 2D.

During this period the EPSC basic properties, namely current decay time constant and ACh equilibrium potential, were stable over time, independent of any EPSC decay. The protective effect of the 250/220-mV cycles was active as long as this sequence was continuously applied, but vanished when this procedure was discontinued, returning to a constant holding potential. At this time the expected synaptic rundown started to develop with the usual time course (not shown).

Determinants controlling EPSC amplitude over time

The EPSC rundown observed while the neuron was held at constant membrane potential occurred only if the preganglionic low rate stimulation was maintained throughout. In the experiment illustrated in Fig. 2E (mean values from 4 neurons), the initial EPSC amplitude was estimated with a single cycle of the
The 0.05-Hz stimulation at −50-mV constant holding potential is resumed after the resting period (mean values from 4 neurons). Bars indicate SE.

The origin of the synaptic decay was investigated in further experiments testing ionic and voltage effects. The presence of nutrients in the bath, the absence of atropine, the external calcium concentration varying in the 2- to 5-mM range, a higher holding potential level (up to −80 mV) or reduction of preganglionic stimulation to 0.03 Hz all proved ineffective in preventing development of the EPSC rundown.

The ionic currents generated during the depolarizing commands (and the resulting intracellular messages) were possibly involved in this unexpected behavior; in fact, activation of all the voltage-dependent currents described in the rat sympathetic neuron starts at −30 mV. In Fig. 3A the sodium and calcium currents associated with the −50/−20-mV command in Fig. 2D were evoked 10 ms before or immediately after the EPSC by applying a single 8-ms voltage step to −20 mV to a neuron held at −50 mV. The timing of Na⁺ and Ca²⁺ injection was only slightly modified with respect to the original protective protocol, but this was sufficient to allow the development of a progressive decrease in EPSC amplitude. The importance of depolarization occurring while the synaptic channels were open (and the absence of any immediate link between activation of ionic voltage-dependent currents and EPSC rundown) was confirmed in the experiment illustrated in Fig. 3B; the −50/−20-mV command cycles preventing the EPSC change in Fig. 2D were precisely reproduced, but preganglionic stim-
ulation was no longer applied within the voltage step but, rather, 10 s before. This canceled the otherwise preventive action of the protocol on EPSC amplitude. The protective effect on synaptic efficacy associated with membrane depolarization, on the other hand, presented an evident voltage threshold for onset of synaptic depression, because recurrent $250/-30$-mV cycles proved inadequate to prevent EPSC rundown (Fig. 3C). Each distinct behavior, illustrated in Fig. 3, A–C, for single neurons, was confirmed in at least three other cells.

The reversibility of EPSC decay was tested, at different times during its development, by applying the $250/-20$-mV protocol, which would have prevented synaptic rundown if used early on. The most frequently observed result was the blocking of any further synaptic change, but no reversal of the effect of depression was seen during subsequent stimulation (an example is given in Fig. 3B). Only occasionally was this procedure alone able to generate some recovery in the EPSC amplitude (Fig. 3D), and only in 1 neuron of 11 did the recovery prove complete.

These data suggest that fast calcium movements in the postsynaptic membrane and external calcium concentrations larger than 2 mM are apparently irrelevant in controlling EPSC amplitude stability over time. The opposite held true when the actual intracellular calcium concentration was modified. Figure 4Aa shows an experiment during which 170 mM BAPTA diffused from the microelectrodes positioned inside the cell.

**Fig. 3.** Relationship between timing of ACh application and neuron depolarization. A: effect on EPSC peak amplitude of short depolarizing pulses ($-50/-20$ mV, 8 ms duration) applied immediately before (initial portion of the recording; see panel) or after complete development of each EPSC evoked by preganglionic 0.05-Hz stimulation in a neuron constantly kept at $-50$-mV holding potential. B: standard $-50/-30$-mV cycles, with command pulses of 100 ms duration, are recurrently applied to a neuron (initial part of the experiment) with preganglionic stimuli no longer occurring during the command pulse but 10 s earlier; thereafter (starting from dashed line), the EPSCs are now evoked during the voltage steps of $-50/-20$-mV cycles (points indicate the corresponding EPSC amplitudes at the 4 different membrane potentials tested). C: recurrent $-60/-30$-mV cycles decrease but are unable completely to prevent synaptic rundown. Arrow indicates the application of a preganglionic tetanus (20 Hz, 10 s duration). D: the synaptic rundown (the inward synaptic charge displacement is indicated here) develops during low-frequency stimulation with the neuron maintained at constant $-50$-mV holding potential. Thereafter (1st vertical dashed line; see panel), recurrent $-50/-20$-mV cycles are started with partial recovery of synaptic current amplitude, which becomes (transiently) complete following a preganglionic tetanus (20 Hz, 10 s duration; 2nd dashed line and arrow).
The effects of this treatment are illustrated in Fig. 4. Ab in a different neuron held at -50 mV under current-clamp conditions. The fast calcium buffer actually reached an internal concentration sufficient to cancel the effects of both the potassium calcium-dependent currents operating in the sympathetic neuron during the action potential: $I_{KCa}$ (which mainly sustains the fast spike repolarization when the cell is maintained at -50 mV) (Belluzzi and Sacchi 1991) and $I_{AHP}$ (which sustains the long-lasting spike afterhyperpolarization) (Sacchi et al. 1995). Under BAPTA treatment, presynaptic stimulation resulted in a progressive decrease in EPSC amplitude, despite the -50/-20-mV voltage cycles (the behavior shown in Fig. 4Aa was confirmed in 4 different neurons). Moreover, under these conditions there was no indication that the basic properties of the synaptic current were consistently affected during the accumulation of the buffer within the postsynaptic neuron.

The 0.05-Hz preganglionic stimulation frequency, used to test synaptic efficacy itself, seems unlikely to contribute significantly to the genesis of any synaptic potentiation. The effects on synaptic strength were, however, so unexpected as to suggest the use of the protective protocols even at higher frequencies. The experiment illustrated in Fig. 2D was thus repeated simply by increasing preganglionic stimulation at 1 Hz in the case of constant holding potential, whereas the protective 0.05 Hz -50/-20-mV cycles were completed with the 1-Hz rate within the interpulse intervals at -50 mV holding potential (thus maintaining the overall 1-Hz rate throughout).

The -50/-20-mV cycles systematically started with the -20-mV pulse, so that the -50-mV/EPSC was the fourth of the sequence; accordingly, the fourth EPSC of the pure 1-Hz series (constant holding potential) was used as the initial reference value. The results of this experiment are illustrated in Fig. 4B. As in the case of the 0.05-Hz stimulation rate, the EPSC peak amplitude (and synaptic charge transfer) rapidly decreased to 43% of the initial values after 5 min stimulation when the holding potential was kept constant, whereas the -50/-20-mV cycles proved to exert the protective effects on synaptic strength under these conditions as well. Point-by-point comparisons between the two experimental groups indicated a significant difference ($P < 0.05$) after 80 s of 1-Hz stimulation ($P < 0.001$ after 2 min). Despite the use of the protective voltage-clamp protocol, the EPSC peak amplitude actually decreased by ~10% during the first minute of stimulation, presumably reflecting the adjustment of ACh release at the new rate; this level, however, was thereafter maintained constant. Notably, the net effect on EPSC depression at 1 Hz was of the same size as that observed at 0.05 Hz.

**Posttetanic effects are influenced by previous stimulation modality**

Potentiation of the fast EPSC was induced by standard afferent tetani at 20 Hz lasting 10 s. Posttetanic effects were systematically observed in all neurons tested, but their devel-
Development was strongly affected by the modalities used during pretetanic stimulation, as illustrated in Fig. 5 in two typical recordings. Preganglionic tetanization was applied in two groups of neurons after a 14-min period of 0.05 Hz supramaximal stimulation, either while maintaining the postsynaptic membrane potential at a constant $-50$-mV level, or during repetitive $-50/20$-mV cycles. The results of these experiments are illustrated in Fig. 6, with peak EPSC amplitudes normalized to the initial values, for clarity. The expected synaptic rundown developed during constant holding potential stimulation, as previously shown in Fig. 2; the tetanus induced a potentiation of the EPSC peak amplitude whose overall magnitude in 10 neurons was $22.8 \pm 5.8\%$ of the pretetanic values (Fig. 6, ●; the difference between the final part of the pretetanic and the initial part of the posttetanic curve is statistically significant, $P < 0.01$). The maximum effect was immediate in four cells and developed over a period of 3–6 min duration in the others; thereafter, the EPSC potentiation started to decline (Figs. 5A and 6). In a group of six neurons, which could be recorded for a sufficiently long time, posttetanic potentiation was virtually concluded after $\sim 40$ min of 0.05 Hz stimulation at $-50$ mV with the return of the EPSC amplitude to the levels that would have been reached in the absence of the tetanic episode. No significant correlation was detected in these experiments between the level of EPSC amplitude depression during the pretetanic stimulation period versus posttetanic recovery. It is worth noting that the original EPSC values observed at the beginning of low-frequency stimulation were not resumed despite the preganglionic tetanization. If the intracellular calcium concentration was controlled by BAPTA (Fig. 4A), the posttetanic potentiation was not evoked and EPSC decayed independent of tetanus and of the protective $-50/20$-mV cycles. Quite a different description applies to the neuron sample in which the EPSC amplitudes were kept con-
stant by application of 0.05-Hz preganglionic stimuli at different membrane potential levels within the repeated −50/−20-mV command cycles. Under these conditions the posttetanic effects were much less evident than in the previous case: in five neurons a short-lived EPSC potentiation of ~4–5% at −50 mV over the pretetanic (and initial) values was observed, after which EPSC amplitude returned to the pretetanic levels and remained constant, indefinitely, during further low-rate stimulation. Statistical analysis on pooled data, however, was unable to detect any significant effect related to the tetanic episode. The results of these experiments are reported in Figs. 5B and 6 (in Fig. 6, open circles represent the mean values of the −50-mV/EPSCs, n = 12; small filled circles illustrate the mean EPSC amplitude time course in 4 neurons, in which the 0.05-Hz sequence at constant holding potential was not intermingled with preganglionic tetanization).

**DISCUSSION**

The new findings presented in this study are related to how constant synaptic strength can be preserved at an intact and mature ganglionic synapse and the different effects of presynaptic tetanization when synaptic efficacy is either depressed or maintained. Depression is shown to occur during slow rate preganglionic stimulation (0.05 Hz) with voltage constantly clamped near resting membrane potential, but it does not occur if the neuron is held at −50 mV without synaptic stimulation, nor in the preparation out of the animal or denervated in the animal for 2 h before experiment. Depression is also prevented during preganglionic stimulation at the same slow rate, but including every 80-s stimulation while the postsynaptic cell is held at −20 mV (as part of −20, −30, −40, −50-mV cycles). Posttetanic potentiation apparently occurs if synaptic currents are depressed and hardly at all if currents are not depressed.

In these voltage-clamp experiments on the rat superior cervical ganglion, however, it is hard to evaluate the true relevance of the posttetanic effects, because there is no precise definition of either the fast EPSC amplitude to be considered as a reference value or of the procedures to determine any previous ganglion potentiation at the time of the dissection and the modalities for its cancellation. Assuming that the EPSC amplitude (or inward charge displacement), measured at the first synchronous preganglionic stimulation of the isolated ganglion, indicates the maximum strength of ganglionic transmission, then the natural conclusion to be drawn from these experiments is that synaptic potentiation following a tetanic episode is very limited or absent. This is in contrast with previous observations in the same preparation based on extracellular or current-clamp recordings (Briggs and McAfee 1988; Briggs et al. 1985). If the EPSC amplitude immediately preceding tetanization is considered as the reference value, then it should be argued that tetanus results in a potentiation of the depressed ganglionic EPSC of ~20%. This is probably the result that most closely fits the general description of LTP in other sympathetic ganglia, although we did not observe long-term maintenance of this effect. EPSC amplitudes higher than the initial ones were only occasionally observed in the present experiments; this suggests that maximal potentiation of nictinic synaptic transmission was already present in the silent
preparation at the moment of its dissection from the animal, and that any subsequent treatment in vitro was, under the most favorable conditions, only adequate to preserve it from failing. The loss in synaptic efficacy was not related to the possible deterioration of the ganglion in vitro, but simply to the modalities of its stimulation or, more precisely, the modalities by which endogenous ACh is applied to the postsynaptic receptor, which is apparently sensitive to the actual transmembrane potential present at the moment of its activation. If the synaptic channels are opened at holding potentials kept constantly close to the presumed resting values, a failure in synaptic transmission progressively ensues, which rapidly and permanently reduces the EPSC amplitude by \(-50\%\). This holds true over a wide range of stimulation frequencies, from the very low ones used to prevent the occurrence of any form of potentiation, to those high enough to mimic the physiological discharge rate present in preganglionic sympathetic fibers at the moment of dissection (reviewed by Jä nig 1995).

The mechanisms involved in the permeation of the ganglionic nicotinic channel therefore appear to be of more general interest than the effects on posttetanic potentiation, which are presumably limited or absent at this synaptic station in which synaptic transmission physiologically occurs with a high safety factor (Sacchi and Perri 1973).

Our results demonstrate that the block of synaptic depression is apparently calcium dependent, but they do not demonstrate how or where Ca\(^{2+}\) acts to be effective. The arguments are the following: 1) BAPTA present in the postsynaptic neuron prevents the protective effects on depression of the depolarizing cycles; 2) depolarizing steps to \(-30\) mV only, prevent synaptic decay less effectively than do steps to \(-20\) mV (most likely due to less activation of calcium channels); 3) depression is not dependent on extracellular [Ca\(^{2+}\)] above 2 mM; and 4) timing of calcium movements is essential, i.e., synaptic channels must be open during depolarization. Depolarizing steps to \(-20\) mV applied 10 ms before or after stimulation are not effective. Full voltage cycles separated from ACh release by 10 s are also ineffective. These results suggest that a postsynaptic increase in [Ca\(^{2+}\)], may not be sufficient by itself to prevent depression at this synapse: rather they indicate that there is an interaction of the effects that occur at depolarized potential (in particular as concerns calcium influx) and the channel characteristics. Hence maximum synaptic conductance apparently occurs in the presence of a calcium-dependent mechanism (e.g., the depolarization-dependent release-promoting factor S coupling spatial facilitation in frog axon terminals (Dudel et al. 1993), or a calcium-dependent polyamine unblock (Rozov et al. 1998)), which requires depolarization simultaneously with the opening of the synaptic channels. Such a mechanism may well be dependent on very close colocalization of calcium channels and synaptic channels and thus may not occur in nonsynaptic patches.

We have no direct data on the basic properties of single channel behavior, except as concerns its mean open time and the equilibrium potential of the permeating cationic current, both of which appear to be unaffected by pre- and posttetanic events. Apparently, the synaptic channels best operate in the presence of recurrent voltage shifts moving the neuron membrane potential toward the ACh equilibrium potential. These are actually the physiological conditions encountered during normal synapse function: the synaptic current depolarizes the cell until an action potential is fired, but the synaptic channels remain open during the depolarizing voltage migrations because of their slow closing kinetics (which, in this preparation, are scarcely influenced by voltage) (Sacchi et al. 1998). If this peculiar coexistence between channel opening, its ionic permeation and depolarization of the membrane hosting the receptor is not obeyed, the synaptic current is expected to rapidly decrease over time in response to constant amounts of neurotransmitter. These findings are reminiscent of previous demonstrations, obtained under current-clamp conditions, in which a precise timing between pre- and postsynaptic activity proved crucial in controlling synaptic strength. When back-propagating postsynaptic spikes were summed with subthreshold EPSPs, LTP of subsequent single EPSPs was produced. Moreover, to strengthen the synapse, EPSP must precede the spike by an interval of 10 ms, or less, whereas synaptic depression is induced when this order was reversed (Markram et al. 1997). A similar conclusion arises from the present experiments under voltage-clamp conditions, in which ACh is applied in the presence or absence of membrane potential voltage jumps. The previous observations were obtained in neocortical slices and confirmed in different preparations including hippocampal CA3-CA3 cell pairs in cultured slices (Debanne et al. 1998), Xenopus retinotectal synapses (Zhang et al. 1998), and cultured hippocampal pyramidal neurons (Bi and Poo 1998). In these preparations N-methyl-D-aspartate (NMDA) receptors are involved in synaptic transmission; therefore the voltage-dependent unblock of NMDA receptors during spike depolarization, and the subsequent Ca\(^{2+}\) influx larger than that contributed by the separate spike and EPSP in isolation (Koester and Sakmann 1998; Yuste and Denk 1995), appear a convincing explanation for LTP. These arguments, however, hardly apply to the nicotinic receptor, in which voltage-dependent block or unblock has never been described. They also provide some support to the contention that the present findings are possibly related to the single channel properties of the nicotinic receptor, rather than to intracellular messages mediated by depolarization.

These properties are potentially of some interest in the organization and maintenance of neuronal functioning. It could be the case of a neuron in which the presynaptic input is normally developed and physiologically active at normal rates, but the neuron itself somehow becomes unable to discharge action potentials; or a neuronal network developed in culture, in the absence of stable pre- and postsynaptic spiking activity. Under these conditions, synaptic transmission would deteriorate through an unknown, merely use-dependent postsynaptic mechanism. The activity-dependent links between postsynaptic versus presynaptic events are well known; in the present example, however, the absence of spike discharge in the postsynaptic neuron would be the main trigger generating the synaptic fading. Similarly well understood is the relief of blocked NMDA-type receptors operated by depolarization; in the ganglion, this principle is apparently reversed, because it is no longer the depolarization that removes the block of the synapse but rather the absence of depolarizing episodes that induces its appearance. This new status is difficult to counteract, at least under the present experimental conditions in the isolated preparation. A possible instrument for recovering initial strength could actually be preganglionic tetanization, which would not represent a functionally relevant modification of
the ganglionic synapse, but simply an attempt to recover the synaptic power lost during inappropriate functioning of the synaptic machinery.

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