

Differences in Ca^{2+} Channels Governing Generation of Miniature and Evoked Excitatory Synaptic Currents in Spinal Laminae I and II

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Many neurons of spinal laminae I and II, a region concerned with pain and other somatosensory mechanisms, display frequent miniature “spontaneous” EPSCs (mEPSCs). In a number of instances, mEPSCs occur often enough to influence neuronal excitability. To compare generation of mEPSCs to EPSCs evoked by dorsal root stimulation (DR-EPSCs), various agents affecting neuronal activity and Ca^{2+} channels were applied to *in vitro* slice preparations of rodent spinal cord during tight-seal, whole-cell, voltage-clamp recordings from laminae I and II neurons. The AMPA/kainate glutamate receptor antagonist CNQX (10–20 μM) regularly abolished DR-EPSCs. In many neurons CNQX also eliminated mEPSCs; however, in a number of cases a proportion of the mEPSCs were resistant to CNQX suggesting that in these instances different mediators or receptors were also involved. Cd^{2+} (10–50 μM) blocked evoked EPSCs without suppressing mEPSC occurrence. In contrast, Ni^{2+} (≤ 100 μM), a low-threshold Ca^{2+} channel antagonist, markedly decreased

mEPSC frequency while leaving evoked monosynaptic EPSCs little changed. Selective organic antagonists of high-threshold (HVA) Ca^{2+} channels, nimodipine, ω -Conotoxin GVIA, and Agatoxin IVA partially suppressed DR-EPSCs, however, they had little or no effect on mEPSC frequency. La^{3+} and mibefradil, agents interfering with low-threshold Ca^{2+} channels, regularly decreased mEPSC frequency with little effect on fast-evoked EPSCs. Increased $[\text{K}^+]_o$ (5–10 mM) in the superfusion, producing modest depolarizations, consistently increased mEPSC frequency; an increase suppressed by mibefradil but not by HVA Ca^{2+} channel antagonists. Together these observations indicate that different Ca^{2+} channels are important for evoked EPSCs and mEPSCs in spinal laminae I and II and implicate a low-threshold type of Ca^{2+} channel in generation of mEPSCs.

Key words: mEPSCs; EPSCs; LVA Ca^{2+} channels; spinal laminae I and II; spinal dorsal horn; rodent; mibefradil; La^{3+}

Many chemically mediated synaptic junctions feature spontaneous, small excitatory postsynaptic potentials or currents (Fatt and Katz, 1952; Boyd and Martin, 1956; Scharfman and Schwartzkroin, 1988). Such miniature excitatory synaptic currents (mEPSCs) are believed to result from the spontaneous release of small quantities of the excitatory chemical transmitter from presynaptic terminals. Therefore, the frequency of mEPSC occurrence is presumed to relate to factors operating presynaptically (Larkman et al., 1991; Nicholls et al., 1992). The release of chemical synaptic transmitters by action potentials in presynaptic nerve terminals depends on entry of extracellular Ca^{2+} through membrane channels opening at relatively large transmembrane depolarizations (Nicholls et al., 1992; Luebke et al., 1993; Wheeler et al., 1994a). On the other hand, the part played by entry of extracellular Ca^{2+} into presynaptic terminals in the occurrence of mEPSCs is a matter of controversy (Fatt and Katz, 1952; del Castillo and Katz, 1954; Boyd and Martin, 1956; Hubbard, 1961).

Our interest in factors controlling generation of mEPSCs stems from the observation that many neurons of the spinal superficial

dorsal horn (laminae I and II) exhibit spontaneous, small inward current transients at relatively high frequencies (Li and Perl, 1994). In a number of cases, these transient currents occur sufficiently often (10–30 per second) to influence the level of background neuronal excitability. The network of largely small neurons and fine neuronal processes that comprise laminae I and II represent a major termination of fine afferent fibers from the spinal dorsal roots (Ranson, 1913; Earle, 1952; Light and Perl, 1979a). Because activity in some thin afferent fibers of the dorsal roots is intimately associated with pain and temperature sensation, laminae I (marginal zone) and II (substantia gelatinosa) have long been argued to be part of the neural apparatus related to these experiences (Earle, 1952; Pearson, 1952; Christensen and Perl, 1970; Perl, 1984). Therefore, factors influencing neuronal excitability in this part of the spinal cord have putative importance for mechanisms related to these sensory processes. Here, we present observations indicative of differences in the Ca^{2+} channels associated with the generation of excitatory postsynaptic currents of the spontaneous miniature type and those evoked by impulses in presynaptic fibers.

Parts of this material have been previously reported (Bao et al., 1995, 1997).

MATERIALS AND METHODS

Preparation. Slices of the rodent spinal cord were prepared as previously described (Li and Perl, 1994). Briefly, young (3–4 week) free-ranging Syrian golden hamsters or Sprague Dawley rats were deeply anesthetized by urethane (1.5 gm/kg i.p.) and cooled on ice to a core temperature below 25°C. The spinal column from the sacral to the midthoracic level was rapidly removed from the areflexive animal, euthanizing it. The spinal cord with associated dorsal roots on one side was quickly freed from the surrounding bone and placed in ice-cold, sucrose-substituted,

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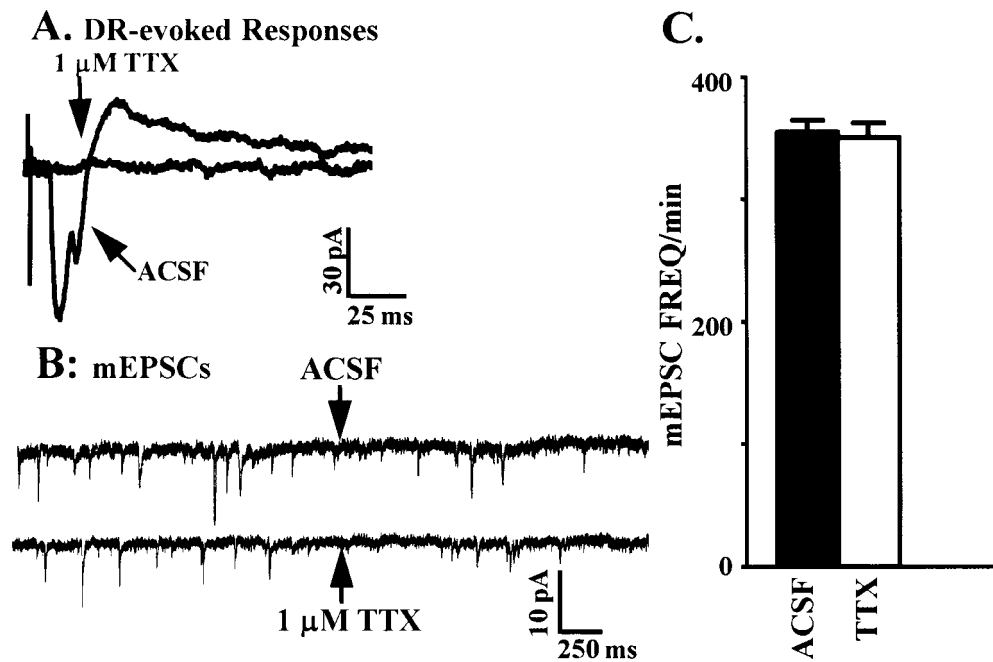


Figure 1. Effects of TTX on evoked and spontaneous mEPSCs. Tight-seal, whole-cell recording from a lamina II_o neuron in a transverse spinal cord slice before and during exposure to 1 μ M TTX. Voltage-clamp mode; inward current is indicated by downward deflection; standard K^+ internal pipette solution. *A*, Average of 10 consecutive responses evoked by electrical pulse stimulation of the ipsilateral, segmental dorsal root repeated at 5 sec intervals (DR-evoked). Initial component of inward current judged to be monosynaptic based on the stability of its latency to near threshold stimuli (data not shown). Illustrated responses were initiated by superthreshold stimuli; inward and outward current components could be dissociated by adjusting the intensity of the DR stimulus. *B*, Sample analog records of mEPSC activity under control conditions (ACSF) and during superfusion with 1 μ M TTX (bottom). *C*, Number of mEPSCs per minute (\pm SEM) of a 5 min continuous recording with standard superfusion solution (ACSF) and with ACSF containing 1 μ M TTX. Error bars indicate SEM. See Materials and Methods for additional details.

artificial CSF (sucrose ACSF) saturated with 95% O_2 and 5% CO_2 (sucrose ACSF, in mM: 234 sucrose, 3.6 KCl, 2.5 CaCl, 1.2 MgCl, 1.2 NaH_2PO_4 , 25 $NaCO_3$, and 12 glucose). The lumbrosacral spinal cord was sectioned into 700–1000 μ m transverse slices in ice-cold sucrose ACSF with a vibratome, taking special care to preserve dorsal root connections. The slices were then incubated for a minimum of 1 hr at room temperature (23–25°C) in “standard” ACSF (in mM: 125 NaCl, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 1.25 NaH_2PO_4 , 26 $NaHCO_3$, and 25 glucose) equilibrated with 95% O_2 and 5% CO_2 .

Electrophysiology. Laminae I and II are readily recognized as a gray band in the superficial part of the spinal dorsal horn (SDH). Recording electrodes were positioned under direct microscopic vision into it. Using the blind patch technique (Blanton et al., 1989), tight-seal, whole-cell recordings were obtained from neurons of the SDH using 4–8 M Ω electrodes filled with either a cesium internal solution (in mM: 130 Cs-gluconate, 5 NaCl, 1 CaCl₂, 1 MgCl₂, 11 EGTA, 10 HEPES, 4 Na-ATP, and 20 tetraethylammonium) to eliminate K^+ currents, or for most of the observations reported, a potassium solution (in mM: 130 K^+ D-gluconic acid, 5 NaCl, 1 CaCl₂, 1 MgCl₂, 11 EGTA, 10 HEPES, and 4 Na-ATP). An electrometer (Axopatch 1-D; Axon Instruments, Foster City, CA) was used to amplify and condition the signals. Recordings were judged to be from neurons by (1) the appearance of large capacitive transients after rupture of gigaohm seals, (2) the presence of spontaneous synaptic potentials, and (3) the generation of action potentials by depolarization under current-clamp recording conditions. “Resting” transmembrane potential at the beginning of a recording session from a neuron typically was –50 to –60 mV. Observations reported herein were largely collected under voltage-clamp conditions at a holding potential of –60 mV using the “potassium” internal electrode solution. A set of observations from a neuron took from 30 to 180 min. The input impedance of the neurons studied ranged from 200 M Ω to >1 G Ω .

Segmental dorsal rootlets of the segment were stimulated with brief pulses (0.2–0.5 msec) using a suction electrode. Observations on dorsal root (DR)-evoked responses were usually made at two reproducible intensities of stimulation, one near threshold and another substantially above threshold (e.g., 2 times); all of those presented or illustrated in this report were supraliminal in intensity.

An analog-to-digital interface (DigiData 1200; Axon Instruments) in an MS-DOS microcomputer was used to digitize and analyze the output from the electrometer using the pClamp6 program. A parallel analog record was stored on magnetic tape using a modified video cassette recorder. The frequency of miniature synaptic events was determined

using a transient event capture and pattern recognition program based on the system described by L. Schmittroth (Bessou and Perl, 1969). Each digitized transient was inspected visually and sorted on the basis of shape and duration. The mean amplitude and size distributions of miniature events were compared before and after experimental manipulations to evaluate whether changes in the frequency could have reflected disappearance of small events in noise. Evoked synaptic potentials were compared principally by the mean of peak amplitudes; most of the data illustrated and described represented the mean of 10 consecutive responses initiated by stimulation at 5 sec intervals. Frequency of miniature events was determined from 1 min periods taken during the second to fifth minute of 5 min samples. Values for mEPSC frequency shown in illustrations, tables, and text represent the mean (\pm SEM) of these four 1 min segments. Differences between mean values of response amplitude or miniature frequency were evaluated using Student’s *t* test.

Agents were added to the standard ACSF superfusion fluid, adjusting osmolarity when necessary. ω -Conotoxin GVIA was obtained from Alamone Laboratories (Jerusalem, Israel) or as a gift from Neurex Corporation (Menlo Park, CA). ω -Agatoxin IVA was a gift from Central Research Division of Pfizer (Groton, CT). Ro 40–5967 was graciously supplied by Hoffman-LaRoche (Berne, Switzerland). Tetrodotoxin (TTX) and metallic compounds were obtained from Sigma (St. Louis, MO). Research Biochemicals (Natick, MA) was the source of all other organic compounds.

The observations were based on detailed study of 141 neurons recorded from laminae I and II; 135 were from hamster, and six were from rat spinal cord slices. The recording locations were always visually verified to be in the gray translucent region forming laminae I and II. For many neurons the visual location was confirmed by intracellular labeling with a fluorescent dye or biotin in the recording pipette and subsequent identification of the labeled neuron and its location in histological preparations made from the spinal cord slices.

RESULTS

Evoked responses

Short-latency (3–40 msec) responses to single pulse stimulation of the attached ipsilateral DR were recorded from almost all (135 of 141) neurons. Commonly, DR-evoked responses were largely excitatory (inward) postsynaptic currents (EPSCs). Some DR-evoked responses appeared as various combinations of inward

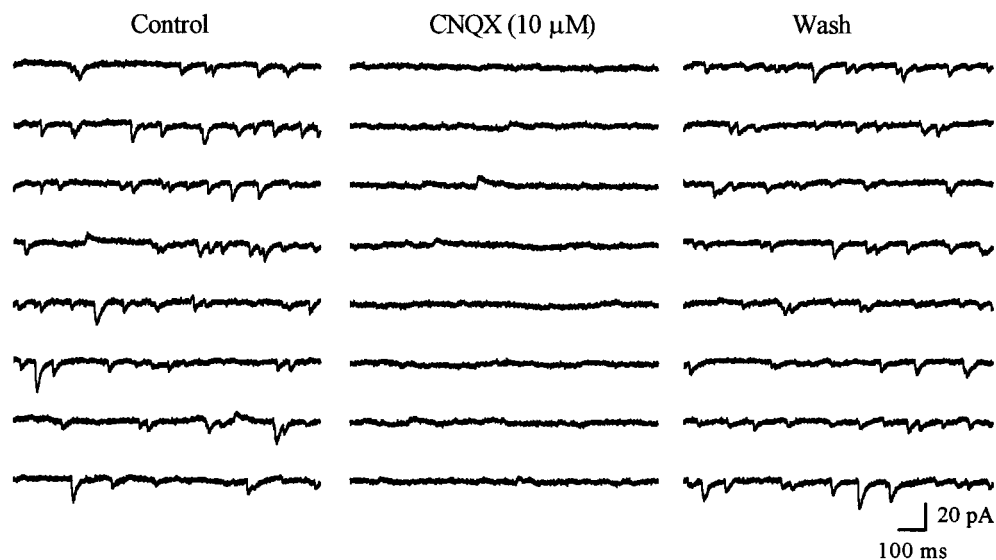


Figure 2. Reversible suppression of mEPSCs by an AMPA/kainate glutamate receptor antagonist (CNQX). Tight-seal, whole-cell, voltage-clamp recording from a lamina I-II_o neuron. Each vertical column displays a continuous record (from top down) of ongoing miniature currents recorded during an 8 sec period. *Control*, Superfusion with standard ACSF. *CNQX*, Recording toward the end of a 4 min superfusion of ACSF containing CNQX (10 μM). *Wash*, Recording after 5 min of superfusion with standard ACSF after the exposure to CNQX, showing partial recovery of mEPSC occurrence. See Materials and Methods for additional details.

and outward currents (Fig. 1A) that in most instances were differentially related to the intensity of the DR stimulus. A number of neurons had phases of DR responses judged to be monosynaptic on the basis of stable latencies to near-threshold stimuli (coefficient of variation $<2\%$, Li and Perl, 1994). In most recordings, the DR-evoked response to suprathreshold stimuli had several inward current peaks. Often the first phase or peak had the stable latency of a monosynaptic connection with later peaks varying in latency as expected for polysynaptic connections. Delayed response phases in certain neurons had the stable latencies of a monosynaptic link (presumably from more slowly conducting DR fibers). A small number of cells exhibited essentially only outward currents to DR stimulation. In the majority of cases, the DR-evoked responses, regardless of form, systematically graded in amplitude and duration as a function of the amplitude or duration of the pulse stimulation. Exceptionally, the evoked response did not increase in amplitude above threshold levels and fluctuated from stimulus to stimulus in an all or none manner. The positive gradation of response with increasing intensity of DR stimulation was interpreted as the effect of recruitment of additional dorsal root fibers to the afferent volley by stronger stimuli and thereby increased excitatory convergence. Conversely, the absence of increased response on stronger DR stimulation was taken to indicate limited convergence. Whether the latter represents a feature of the connectivity to certain neurons or reflects partial denervation secondary to the preparation of a slice was not established. The reported observations were made on neurons in which DR-evoked EPSC showed progressive gradation for increasing DR stimulus intensity. Comparisons of the evoked responses were made on DR-evoked inward currents to a particular suprathreshold stimulus.

The AMPA/kainate glutamate receptor antagonist, CNQX (6-cyano-7-nitroquinoxaline-2,3-dione), at concentrations of 10 μM, regularly greatly reduced or completely abolished the short-latency DR-evoked EPSCs. When this concentration produced only partial block of the evoked response, increasing the CNQX concentration to 20 μM suppressed the remaining evoked activity. These observations confirm previous conclusions that DR stimulation evokes short-latency (<40 msec) excitatory responses in superficial dorsal horn neurons that are principally or exclusively mediated by AMPA/kainate glutamate receptors (Yoshimura

and Jessell, 1990; Li and Perl, 1995). Some neurons also showed slow, delayed (>50 msec) responses that were not suppressed by CNQX. These late responses were abolished ($n = 5$) by the NMDA receptor antagonist AP-5 (2-amino,5-phosphopentanoic acid). Our observations and analyses consider only the short-latency responses.

A purine, putatively ATP, is known to have excitatory effects and to open fast-type synaptic cation channels in some neurons of the superficial dorsal horn (Jahr and Jessell, 1983; Fyffe and Perl, 1984; Evans et al., 1992; Li and Perl, 1995; Bardoni et al., 1997). There may be a purine contribution to the short-latency responses evoked in some laminae I and II neurons by stimulation of primary afferent fibers, although the typical complete block of the responses by AMPA/kainate receptor antagonists suggests the glutamate receptor-mediated portion is obligatory for effective, fast synaptic activation (Li and Perl, 1995; Bao et al., 1995, 1997).

Miniature synaptic events

In the absence of stimulation, tight-seal, whole-cell, voltage-clamp records from many laminae I and II neurons exhibit numerous, brief (5–20 msec), irregularly occurring small inward currents. Examples from three different neurons appear in Figures 1B, 2 and 5B. These random background events vary in amplitude considerably (5–50 pA), typically averaging ~ 15 pA at -60 mV holding potential. Under stable recording conditions, the spontaneous miniature events persist for hours with little change in average amplitude or in mean frequency of occurrence. In many neurons they appear more frequently than 10/sec and in a number of instances exceed 25/sec. TTX, at concentrations abolishing the DR-evoked EPSCs, had a negligible effect on the average frequency (or mean size) of the small background currents (Fig. 1, Table 1A). Larger (>30 pA) random currents were relatively rare (<10 in 60 sec) and highly variable in occurrence. In some neurons the number of such larger spontaneous events appeared to decrease in the presence of TTX, but this proved difficult to document because of their rarity and irregularity.

The spontaneous inward currents disappeared or reversed in polarity near zero transmembrane potential. The GABA_A and glycine receptor antagonists bicuculline and strychnine had no effect on the amplitude of the miniature inward currents at a

Table 1. Summary of the effects of various agents on mEPSC frequency and the peak amplitude of dorsal root-evoked EPSCs

Agent (number of neurons)	mEPSC frequency	Evoked EPSC amplitude
A		
2 μM TTX (5)	-15.2% (± 5.7)	-100% (± 0)
50 μM Cd^{2+} (5)	+87% (± 49.5)	-90% (± 10)
10 μM Nimodipine (8)	+5% (± 14)	-23% (± 5)
1 μM ω -Conotoxin GVIA	-13% (± 7.3)	-74% (± 5.7)
0.1 μM Agatoxin IVA (6)	+4% (± 16.8)	-36% (± 9)
B		
1 μM La^{3+} (2)	-5% (± 2.5)	+19% (± 3.5)
5 μM La^{3+} (4)	-29% (± 5.8)	-7% (± 5.4)
10 μM La^{3+} (5)	-35% (± 5.8)	-9% (± 6.5)
1 μM Ro 40-5967 (2)	-29% (± 1)	+4.5% (± 9.5)
2.5 μM Ro 40-5967 (6)	-39% (± 7.9)	-3% (± 8.5)
5 μM Ro 40-5967 (19)	-47% (± 3.5)	-7% (± 7.0)
C		
5 mM $[\text{K}^+]_o$ ACSF (4)	+55% (± 7.6)	+3% (± 8.7)
10 mM $[\text{K}^+]_o$ ACSF (15)	+100% (± 14.1)	+8% (± 8.6)
5 mM $[\text{K}^+]_o$ Ro 40-5967 (4)	-14% (± 11)	-15.8% (± 6.6)
10 mM $[\text{K}^+]_o$ Ro 40-5967 (5)	-15% (± 8.5)	-10% (± 5.9)

Each neuron was studied in a separate slice, and only one compound modifying the superfusion fluid was tried in a given slice. The number of neurons tested with a given agent or procedure is given in parentheses. mEPSC frequency per minute was determined for the last 4 min in 5 min samples of ongoing activity and expressed as a percentage variation from control values. The change indicated (%) is the test mean divided by the immediately preceding mean control value. "+" indicates an increase over control values (\pm SEM); "-" indicates a decrease compared to control value (\pm SEM). Evoked (superthreshold) EPSC peak amplitudes (10 consecutive responses) take as a reference the mean response amplitude before application of an agent (test/control \times 100). A, Action of TTX and established Ca^{2+} channel antagonists. B, Effects of La^{3+} and mibefradil (Ro 40-5967). C, Effects of $[\text{K}^+]_o$ increases on mEPSC frequency and DR-evoked responses and their modification by 5 μM Ro 40-5967 applied 10 min before.

holding potential of -60 mV. These observations suggest that the great majority of the miniature inward currents did not result from presynaptic action potentials and were not GABA_A- or glycine-mediated. Therefore, most of the small inward currents appear to represent mEPSCs of the type noted in a variety of chemically mediated synapses (Fatt and Katz, 1952; Parfitt and Madison, 1993; Scharfman, 1993; Gottmann et al., 1994; Wyllie et al., 1994).

Laminae I and II form a heterogeneous region with structural and functional differences among the component neurons (Ramon y Cajal, 1909; Réthelyi and Szentágothai, 1973; Kumazawa and Perl, 1978; Light et al., 1979; Cervero and Iggo, 1980; Réthelyi et al., 1989). Therefore, it was not surprising that more than one synaptic mediator appeared to be associated with the mEPSCs in certain neurons. In many neurons 10–20 μM CNQX caused a reversible complete disappearance of the mEPSCs (Fig. 2); however, in other instances this AMPA/kainate receptor antagonist, in concentrations abolishing evoked EPSCs, produced only a reduction of mEPSC frequency (data not shown). NMDA receptor antagonists (e.g., AP-5) were found to be without effect on mEPSC frequency ($n = 5$, data not shown).

At the holding potential of -60 mV some neurons exhibited miniature irregularly appearing outward as well as inward currents. The glycine receptor antagonist strychnine produced notable decreases in the appearance of spontaneous outward currents in the hamster laminae I and II neurons on which it was tested without causing a change in mEPSC frequency or amplitude. Quantification of effects on miniature outward currents was not attempted.

ATP has been shown to induce fast inward currents in laminae

I and II neurons and to be involved in synaptic transmission to at least some neurons of the region (Li and Perl, 1995; Bardoni et al., 1997). Incomplete block of the mEPSCs by CNQX in some neurons suggested that in these instances the miniatures were partially generated by a different transmitter and receptor combination. In several such neurons, the purinergic P₂ receptor antagonist suramin (0.5–1 mM) suppressed the number of mEPSCs, particularly those of smaller amplitude; however, in other instances, mEPSC frequency actually increased after suramin. The effects of suramin were difficult to reverse, and the variability of action limited study of its effects.

These pharmacological evaluations of the miniature inward currents led us to conclude that in the majority of instances they resulted from activation of fast glutamate channels of the AMPA/kainate type by a glutamate transmitter either with, in certain neurons, a possible contribution from a purinergic agent acting on a P_{2x} purinergic receptor or some other mediator-receptor combination.

Effects of divalent metallic ions

As already mentioned, conclusions from the literature are divided on the extent to which the frequency of random miniature excitatory synaptic events depend on extracellular Ca^{2+} (Fatt and Katz, 1952; del Castillo and Katz, 1954; Boyd and Martin, 1956; Hubbard, 1961). In our hands, elimination of Ca^{2+} in the ACSF, without substitution by other divalent ions, regularly caused loss of the whole-cell seal. As shown in Figure 3, substitution of the standard ACSF by an ACSF containing 10 mM Mg^{2+} and no Ca^{2+} caused a sharp drop in mEPSC frequency ($n = 4$) and eliminated the DR-evoked response.

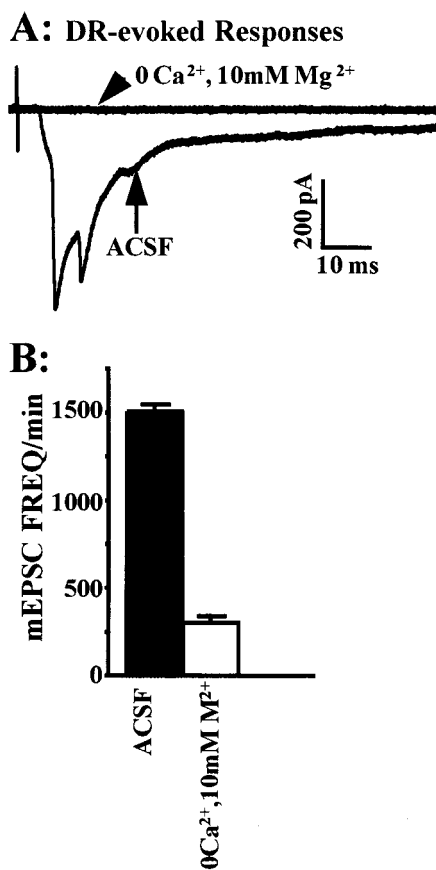


Figure 3. Effects of zero Ca^{2+} and 10 mM Mg^{2+} in superfusing ACSF on DR-evoked and background mEPSCs recorded from a lamina II neuron. *A*, EPSCs evoked by stimulation of the segmental dorsal root (DR-evoked). Average of 10 consecutive DR-evoked responses under control conditions (ACSF) and after 5 min superfusion with ACSF containing zero Ca^{2+} and 10 mM Mg^{2+} . *B*, Average mEPSC frequency during superfusion with standard ACSF and with ACSF containing zero Ca^{2+} and 10 mM Mg^{2+} . See Figure 1 legend and Materials and Methods for additional details.

Addition of Co^{2+} (4–5 mM in HEPES ACSF) and elimination of Ca^{2+} abolished DR-evoked EPSCs and sharply reduced mEPSC frequency (Fig. 4). Co^{2+} is known to interfere with Ca^{2+} currents, and this action suggests that at least part of the process related to the production of mEPSCs depends on extracellular Ca^{2+} entering through plasma membrane channels (Hille, 1992).

A distinction between the Ca^{2+} channels associated with the production of evoked and of miniature synaptic events is suggested by differences in the actions of Cd^{2+} and Ni^{2+} , two other divalent cations established to interfere with such channels. Figure 4 and Table 1*A* illustrate that Cd^{2+} at relatively low concentrations ($\leq 50 \mu\text{M}$) largely or totally suppressed the EPSCs evoked by DR stimulation while increasing mEPSC frequency. The Cd^{2+} -related increase in mEPSC frequency has been previously noted (Li and Perl, 1995) and possibly is a product of Ca^{2+} made available from intracellular stores. Higher concentrations of Cd^{2+} (100–500 μM) severely depressed mEPSC frequency (data not shown). Conversely, Ni^{2+} in concentrations of 20–100 μM left the early monosynaptic phase of the DR-evoked response largely intact (Fig. 5*A*) while substantially reducing mEPSC frequency (Fig. 5*B–C*). These relatively low concentrations of Ni^{2+} usually

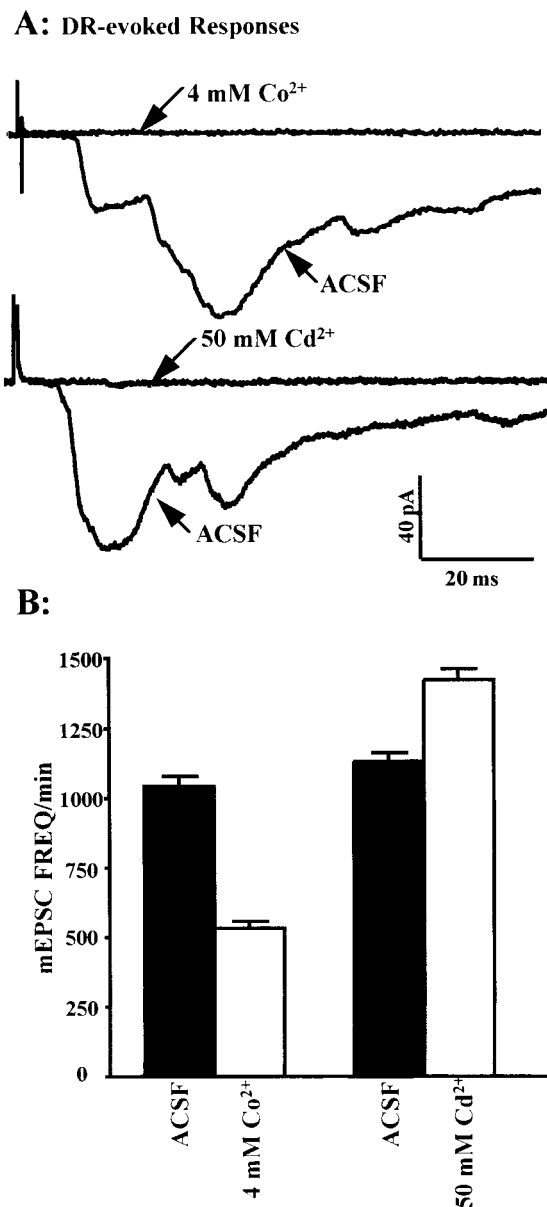
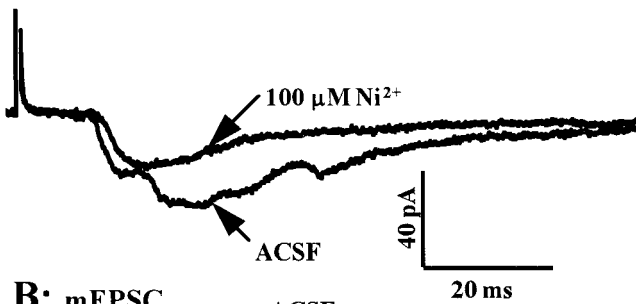


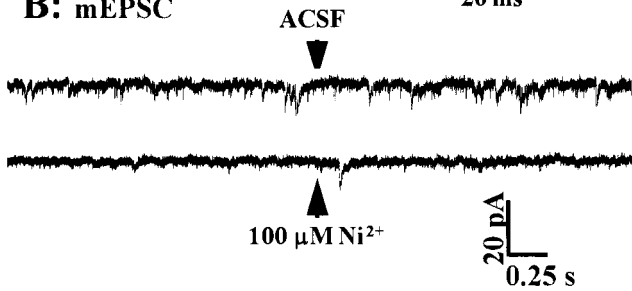
Figure 4. Examples of the effects of Co^{2+} and Cd^{2+} on evoked EPSCs and mEPSCs recorded from laminae I and II neurons. Tight-seal, whole-cell recordings in voltage-clamp mode. Only one agent was used on a given neuron and a spinal slice. *A*, Average EPSCs evoked by 10 successive stimuli at 5 sec intervals to the segmental dorsal root. The evoked responses were obtained at the end of a 5 min sample of ongoing activity for the data in *B*. ACSF, Control conditions using standard superfusion. 4 mM Co^{2+} , Records obtained at end of a 5 min exposure to the Co^{2+} superfusate. 50 μM Cd^{2+} , Records obtained at the end of a 5 min exposure to the Cd^{2+} superfusate. *B*, Average mEPSC frequency during control ACSF superfusion (solid bars) and during exposure to ACSF containing the indicated divalent ion (open bars); same neurons and manipulations as in *A*. The difference between the mean mEPSC frequency for the standard ACSF and for the exposure to divalent cation in both cases had a chance probability of $p < 0.001$ (Student's *t* test). See Figure 1 legend for additional details.

suppressed later, polysynaptic components of DR-evoked responses (Fig. 5*A*). The latter action may be secondary to cumulative effects of successive small depressions of transmission in a multineuronal linkage. The effects of low concentrations of Cd^{2+} and Ni^{2+} on evoked and spontaneous mEPSCs are consistent

A: DR-evoked Responses



B: mEPSC



C:

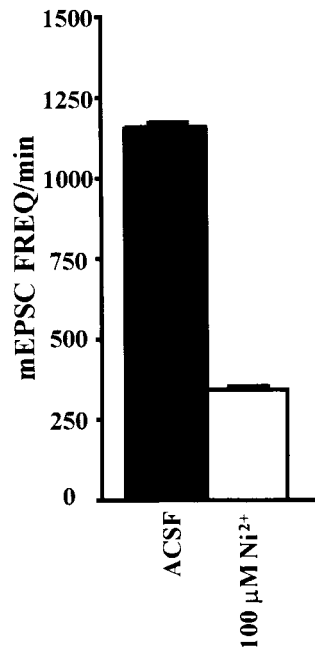


Figure 5. Effects of Ni²⁺ on evoked EPSCs and mEPSCs recorded from a lamina II neuron. *A*, Average of 10 consecutive DR-evoked responses under control (ACSF) and after 5 min superfusion with ACSF containing 100 μM Ni²⁺. The initial inward current phase had the constant latency attributable to a monosynaptic connection. The later phases varied more in latency suggesting a polysynaptic linkage. *B*, Sample analog records from neuron of *A* showing background mEPSC activity under control conditions (ACSF) and during superfusion with ACSF containing 100 μM Ni²⁺ (*bottom*). *C*, Average mEPSC frequency from neuron of *A* during control ACSF superfusion (*solid bar*) and during superfusion with ACSF containing 100 μM Ni²⁺ (*open bar*). Difference between mean values in *C* had a chance probability of *p* < 0.001 (Student's *t* test). See legends for Figures 1 and 4 for other details.

with a difference in the involved varieties of Ca²⁺ channels. Cd²⁺ concentrations under 100 μM are reported to interfere preferentially with high-threshold [high voltage-activated (HVA)] Ca²⁺ channels. The opposite is described for Ni²⁺; Ni²⁺ blocks low-threshold (LVA) Ca²⁺ channels at concentrations that produce minimal effects on high-threshold Ca²⁺ channels of the same cells (Byerly and Hagawara, 1988; Fox et al., 1987). Thus, the Cd²⁺ and Ni²⁺ results suggest that mEPSC generation may be partially governed by opening of a low-threshold type of Ca²⁺ channel.

Selective organic HVA Ca²⁺ channel antagonists

Specific antagonists for one or more high-threshold Ca²⁺ channels suppress release of transmitter by presynaptic impulses at vertebrate central synapses, thereby reducing the postsynaptic response (Luebke et al., 1993; Wheeler et al., 1994a). Such antagonists proved to have little effect on mEPSC frequency in laminae I and II neurons. As Table 1*A* documents, the dihydropyridine nimodipine (10 μM), an established L channel antagonist, produced an insignificant average increase in mEPSC frequency, and on average, was associated with a small decrease (–23%) of DR-evoked EPSC amplitude. The selective N channel-blocking agent ω-Conotoxin GVIA (1 μM) only slightly reduced mEPSC frequency (less than –15%), however, it substantially suppressed (more than –70%) the average DR-evoked EPSC (Table 1*A*). Table 1*A* also shows that the P/Q Ca²⁺ channel antagonist, Agatoxin IVA (0.1 μM), lacked consistent effect on mEPSC frequency but resulted in a moderate decrease of DR-evoked EPSC amplitude (mean more than –30%).

Agents affecting low-threshold (low voltage-activated) Ca²⁺ channels

Unfortunately, antagonists specific for low-threshold (T-type) Ca²⁺ channels have proven elusive, and none were available to us. On the other hand, several agents have been shown to suppress LVA Ca²⁺ currents in a partially selective, concentration-

dependent manner. Amiloride is reported to interfere with T-type Ca²⁺ current in cardiac and neural cells (Tang et al., 1988), however, in the presence of this compound, viability of neurons in our spinal slices was compromised, and the effects on neuronal responses varied widely. La³⁺, along with other trivalent ions in low micromolar concentrations, blocks low-threshold Ca²⁺ currents in excitable cells including central neurons, although at higher concentrations it can have other actions (Mele, 1969; Reichling and MacDermott, 1991; Mlinar and Enyeart, 1993). Figure 6 and Table 1*B* show that La³⁺ at 5–10 μM suppressed mEPSC frequency considerably while causing very little or no reduction of evoked EPSCs. In fact, in some instances exposure to these levels of La³⁺ resulted in increased amplitude of DR-evoked EPSCs in conjunction with a decrease of mEPSC frequency.

The novel compound mibefradil (Ro 40–5967) is described as selectively interfering with T-type currents in vascular smooth muscle (Mishra and Hermsmeyer, 1994). This compound also acts on other Ca²⁺ channels (e.g., R-type), although low-threshold channels appear to be more sensitive to it (Wheeler et al., 1994b; Bezprozvanny and Tsien, 1995; Randall and Tsien, 1997). As shown by the example in Figure 6 and by Table 1*B*, Ro 40–5967, at 1–5 μM, considerably reduced mEPSC frequency in conjunction with only minor and variable effects on the DR-evoked EPSCs. The data from another neuron in Figure 7 illustrate that the suppression of mEPSC frequency by mibefradil was neither associated with significant changes in mean mEPSC amplitude nor in the distribution of mEPSC amplitudes.

Increased [K⁺].

The observations to this point suggest that substances interfering with low-threshold Ca²⁺ channels depress generation of mEPSCs and thereby their frequency of occurrence. If this is the case, it could be expected that relatively small depolarizations should produce increased opening of LVA Ca²⁺ channels on presynaptic

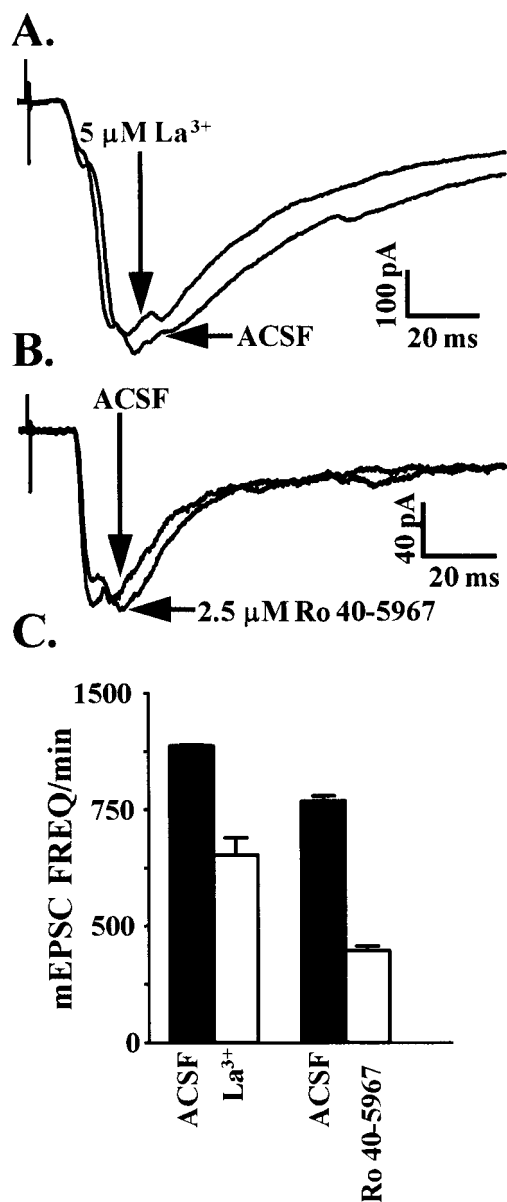


Figure 6. Effects of La^{3+} and mibefradil (Ro 40-5967) on evoked EPSCs and mEPSCs of laminae I and II neurons. *A, B*, Tight-seal, whole-cell recordings in voltage clamp mode from different slices. (Note separate calibrations). EPSCs evoked by stimulation of the segmental dorsal root. La^{3+} ($5 \mu\text{M}$) and Ro 40-5967 ($2.5 \mu\text{M}$) were added to standard ACSF. See legends for Figures 1 and 3 for additional information. *C*, Same neurons as in *A* and *B*. Bars showing mean mEPSC frequency (\pm SEM) for second to fifth minute of a 5 min sample of background activity during superfusion with the standard ACSF (solid bars) and ACSF containing the indicated agent (open bars). Differences between control and test mEPSC frequencies in both instances had chance probability of $p < 0.01$ (Student's *t* test).

terminals and augment entry of extracellular Ca^{2+} . Greater presynaptic intracellular Ca^{2+} in turn could be expected to increase the occurrence of excitatory miniatures. Selective depolarization of fine presynaptic terminals that originate from multiple sources in the complex neuropil of the laminae I and II was beyond our capabilities. As an alternative, we turned to the effect of modestly increasing $[\text{K}^+]_o$ in the superfusing ACSF. Based on estimates of intracellular ionic concentrations and a relative permeability constant for Na^+ of 5% that of K^+ , from the constant

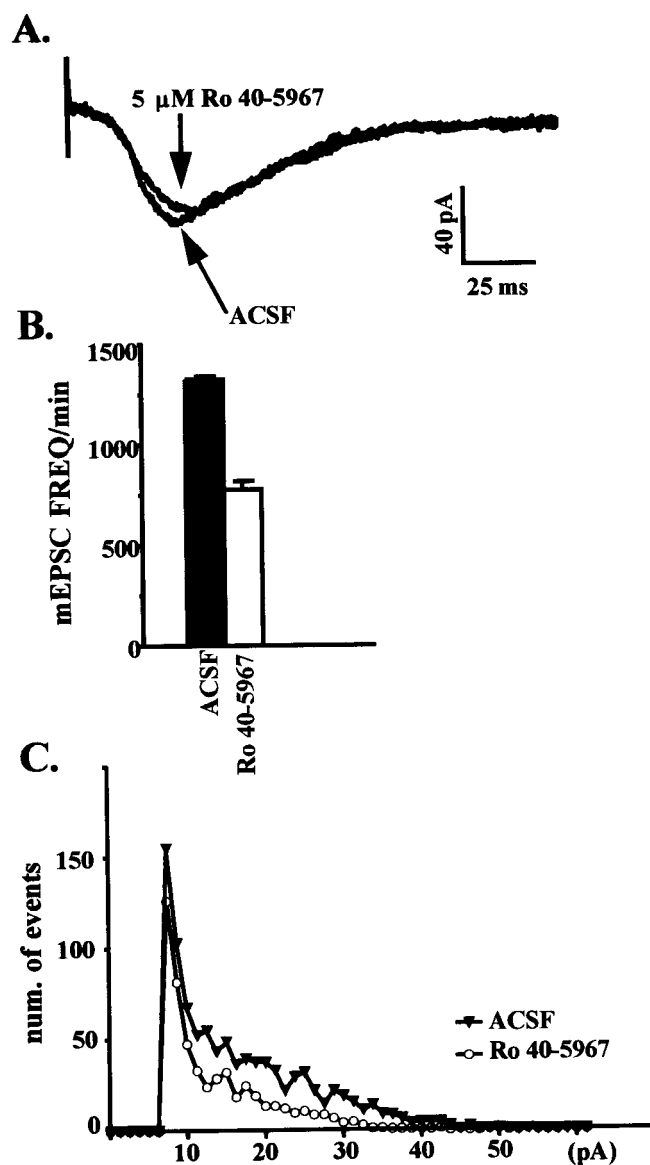


Figure 7. Effects of mibefradil (Ro 40-5967) on background mEPSCs. Tight-seal, whole-cell recording from a neuron in lamina I-II_o. *A*, Average amplitude of mEPSCs during the 4 consecutive min of recording used for the graph of *B*. *B*, Average mEPSC frequency (\pm SEM) during second to fifth minute of 5 min superfusion with the indicated solution. *C*, Amplitude distribution of mEPSCs during 1 min of control superfusion and during 1 min of superfusion with ACSF containing $5 \mu\text{M}$ Ro 40-5967. Same recording as *A* and *B*.

field equation $[\text{K}^+]_o$, increases to 10 mM or less should result in depolarization of neuronal elements of the slice by <20 mV (Nichols et al., 1992).

Increases in $[\text{K}^+]_o$ in the superfusion ACSF above the standard ACSF concentration of 2.5 mM consistently increased mEPSC frequency. The magnitude of the frequency increase varied from cell to cell. The average increase in mEPSC frequency for a change in $[\text{K}^+]_o$ from 2.5 to 5 mM was $\sim 50\%$ ($n = 4$) and, for an increase to 10 mM ($n = 15$) the average miniature occurrence doubled (Fig. 8, Table 1C). These observations imply a positive relationship between $[\text{K}^+]_o$ concentration over the range of 2.5–10 mM and mean mEPSC frequency. During the exposure to 10 mM $[\text{K}^+]_o$ ACSF, postsynaptic cells (in voltage

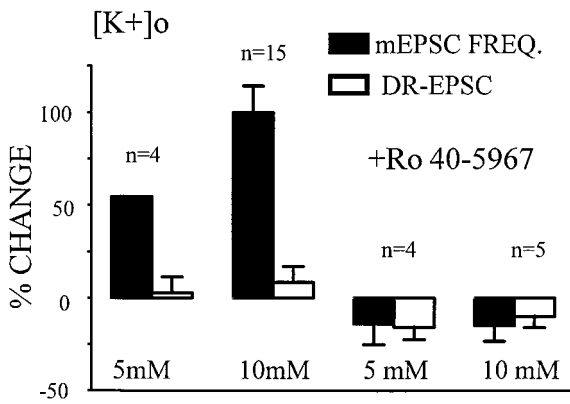


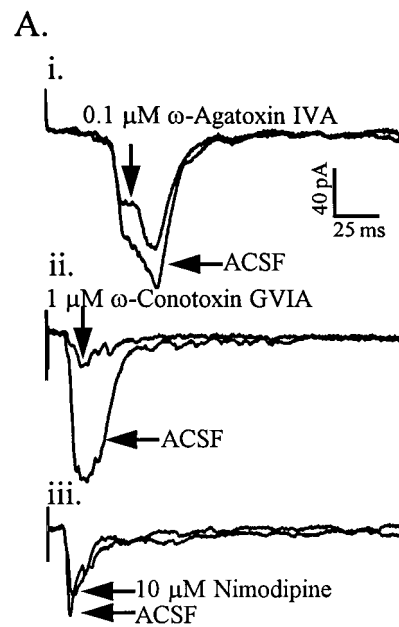
Figure 8. Effects of increased [K⁺]_o on evoked EPSCs and mEPSCs and its block by mibefradil (Ro 40-5967). Each bar indicates the average percentage change from the control level (standard ACSF superfusion). Filled bars, mEPSC frequency per minute. Open bars, Peak amplitude of the DR-evoked EPSC. [K⁺]_o was increased from 2.5 to either 5 or to 10 mM. Right, [K⁺]_o in the superfusion was increased to indicated level 10 min after exposure to 5 μM Ro 40-5967. The n above each pair of bars indicates the number of neurons tested.

recordings) depolarized by <10 mv. Depending on their steady state inactivation curve, this degree of depolarization could increase opening of low-threshold Ca²⁺ channels in small DRG neurons (Scroggs and Fox, 1992).

Increasing [K⁺]_o from 2.5 to 5 (n = 4) or 10 mM (n = 15) produced small, inconsistent changes in DR-evoked EPSCs (Fig. 8, Table 1C) in neurons exhibiting substantial increases in mEPSC frequency. The minimal effect of these increases in [K⁺]_o on the DR-evoked EPSCs suggests that [K⁺]_o changes did not result in important alterations of excitability of either primary afferent presynaptic endings or in the recorded postsynaptic neurons.

If increased low-threshold Ca²⁺ channel opening is related to the enhanced mEPSC frequency, agents interfering with these channels should antagonize the [K⁺]_o action. Ro 40-5967 (5 μM) applied before raising [K⁺]_o to 5 or 10 mM blocked the expected mEPSC frequency increase (Fig. 8, Table 1C). Elevating [K⁺]_o in the presence of Ro 40-5967 produced small to moderate decreases of excitatory miniature occurrence. In the same neurons, the combination of Ro 40-5967 and increased [K⁺]_o led to small or marginal decreases in the amplitude of DR-evoked responses (Fig. 8, Table 1C). It is noteworthy that the increase in mEPSC frequency by exposure to increased [K⁺]_o and its reversal by Ro 40-5967 in rat neurons (n = 6) was indistinguishable to that produced in neurons of hamster slices. The data in Table 1C for these observations of [K⁺]_o manipulation in the presence of Ro 40-5967 pools that obtained from experiments on both species.

In distinction to the Ro 40-5967 action, antagonists of high-threshold Ca²⁺ channels that suppress DR-evoked EPSCs, uniformly failed to block the increase of mEPSC frequency evoked by augmenting [K⁺]_o. Fig. 9 depicts the effects of ω-Agatoxin IVA, ω-Conotoxin GVIA, and nimodipine on mEPSC frequency by themselves and in combination with increases of [K⁺]_o to 10 mM. Thus, “specific” blockers of P/Q (ω-Agatoxin IVA), N (ω-Conotoxin GVIA), and L (nimodipine) high-threshold Ca²⁺ channels, in concentrations effective on other tissues or suppressing evoked EPSCs, appeared neither to inhibit mEPSC frequency nor to reverse a [K⁺]_o-evoked increase in mEPSC frequency. Nimodipine was interesting in that in some cases it resulted in



B:

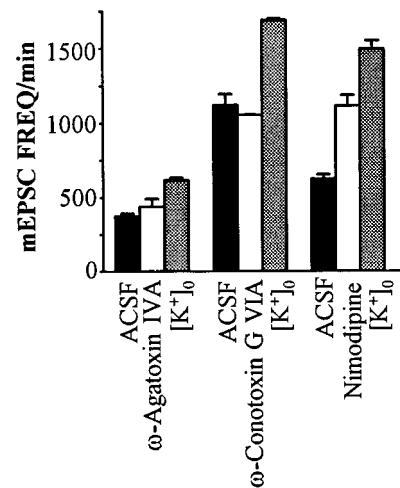


Figure 9. Action of selective antagonists of HVA Ca²⁺ channels on EPSCs in laminae I-II neurons. *A*, Average DR-evoked EPSCs; *i*, *ii*, *iii* are from different spinal cord slices. Calibration in *Ai* applies to all. *B*, Each set of three bars represents observations made on a neuron whose responses are shown in *A* and indicates mEPSC frequency per minute (± SEM) for the last 4 min of 5 min observation periods. Solid filled bars, Control superfusion (ACSF). Open bars, Mean frequency after 10 min application of the indicated agent. Patterned bars, Effect of increasing [K⁺]_o to 10 mM in the presence of the indicated Ca²⁺ channel antagonist. Note: Increase in mEPSC frequency after nimodipine shown was greater than average (see Table 1C for additional information).

substantial increases of mEPSC frequency (Fig. 9), although on the average its effect was minimal (Table 1A).

DISCUSSION

The suppression of the mEPSC frequency in neurons of spinal laminae I and II by low Ca²⁺ and by the divalent cations Co²⁺ and Ni²⁺ argues in favor of a partial dependence of the generation of these miniature synaptic events on entry of extracellular

Ca^{2+} into presynaptic terminals. This conclusion is supported by the inhibition of mEPSC frequency by La^{3+} and mibefradil. All of these extrinsic agents have been found to block voltage-sensitive Ca^{2+} channels in excitable tissues (Hille, 1992; Mlinar and Enyeart, 1993; Mishra and Hermsmeyer, 1994; Bezprozvanny and Tsien, 1995).

The major feature of our study is the diametric differences in the effects of agents established to interfere with Ca^{2+} channels on mEPSC frequency and on the amplitude of EPSCs evoked by presynaptic impulses. This distinction is evident for both ionic agents (Ni^{2+} , Cd^{2+} , and La^{3+}) and organic compounds (ω -Conotoxin GVIA, ω -Agatoxin IVA, and mibefradil). Accepting entry of extracellular Ca^{2+} to be related to the generation of the spontaneous events, the dissociation in actions on the evoked EPSCs and the miniature, ongoing inward currents strongly supports the concept of differences in the nature of Ca^{2+} channels that are involved.

A combination of observations support entry of Ca^{2+} through LVA channels to be a factor in the generation of spontaneous mEPSCs. One indication is the potent depression of miniature frequency by relatively low concentrations of Ni^{2+} and the resistance of their suppression by low levels of Cd^{2+} . Calcium-carried current through low-threshold channels is known to be blocked by the levels of Ni^{2+} that we found to suppress mEPSC frequency (Fox et al., 1987). Conversely, lack of suppression of mEPSC frequency in our hands to levels of Cd^{2+} established to block high-threshold Ca^{2+} channels but not the low-threshold type (Fox et al., 1987; Byerly and Hagiwara, 1988) also is consonant with a role for low-threshold Ca^{2+} channels in the generation of miniature synaptic currents.

A second consideration is the suppression of mEPSC frequency by agents known to interfere with low-threshold Ca^{2+} channels. Unfortunately, in contrast to the situation for some high-threshold Ca^{2+} channels, a highly selective or specific antagonist for Ca^{2+} channels activated at near resting intracellular potentials (e.g., T-type) was not available. Therefore, our experiments aimed at inhibiting Ca^{2+} entry through low-threshold channels were forced to depend on agents such as La^{3+} and mibefradil (Ro-40-5967) that have other actions as well. Both La^{3+} and mibefradil are reported to be effective blockers of T-type channels in certain tissues, but they also affect Ca^{2+} current through high-threshold channels and $\text{Na}^+/\text{Ca}^{2+}$ transport mechanisms (Ikeda et al., 1992; Bezprozvanny and Tsien, 1995; Randall, 1995; Randall and Tsien, 1997). Importantly for the present consideration, although both La^{3+} and mibefradil, in the concentrations used, substantially reduced the frequency of mEPSCs, they did so without producing significant changes in EPSCs evoked by stimulation of dorsal root fibers in the same neurons. Thus, neither agent significantly altered the ability of presynaptic fibers to release transmitter in response to presynaptic impulses. The La^{3+} and mibefradil actions are highlighted by the converse effects produced by selective antagonists for the N (ω -Conotoxin GIVA) and P/Q (ω -Agatoxin IVA) high-threshold channels. Both N and P/Q channel antagonists suppressed evoked EPSCs at concentrations that had little effect on miniature frequency.

Elimination of the high-threshold R-type Ca^{2+} channels as a consideration is more difficult because mibefradil may block this channel at concentrations similar to those antagonizing the low-threshold T-type channels (Randall and Tsien, 1997). Nevertheless, we believe it unlikely that the R-type high-threshold channel is importantly involved in the mEPSC gen-

eration in laminae I and II neurons because of the effectiveness of low concentrations of Ni^{2+} in suppressing mEPSC occurrence.

The effects of small increases in the concentration of K^+ in the superfusion fluid also are consistent with modulation of miniature frequency by Ca^{2+} entry through a low-threshold channel. Increases of $[\text{K}^+]_o$ from 2.5 mM to 5 or 10 mM considerably increased mEPSC frequency. These increases in $[\text{K}^+]_o$ could be expected to produce relatively small depolarizations of presynaptic terminals as well as other neuronal elements in the slice. This interpretation is supported by the reversal of the K^+ -induced mEPSC frequency by mibefradil (Ro 40-5967) but not by selective high-threshold Ca^{2+} channel antagonists. Admittedly, the concept that the potassium depolarization opens low-threshold Ca^{2+} channels to provide additional intracellular Ca^{2+} is open to challenge on the basis of the usual characterization of T-type channels as partially inactivated at our observed membrane potentials. However, our observations were made on the postsynaptic element. The transmembrane potential of the presynaptic terminals is not known. Furthermore, low-threshold Ca^{2+} channels in the spinal dorsal horn neurons may vary from those that have been studied to date in other regions and tissues. There are indications that certain otherwise typical T-type channels do not inactivate as rapidly as is commonly supposed (Hille, 1992; Randall and Tsien, 1997). In any case, in light of the actual postsynaptic measurements, the depolarizations produced by increasing $[\text{K}^+]_o$ to 5 or 10 mM may not result in the broad inactivation of low-threshold Ca^{2+} channels expected from other circumstances for certain T-type channels (Fox et al., 1987; Scroggs and Fox, 1992; Randall and Tsien, 1997).

These considerations lead us to propose that external calcium entry through low-threshold channels is at least partially responsible for the release of transmitter that generates miniature excitatory postsynaptic currents in neurons of the superficial dorsal horn of the spinal cord. This does not imply that Ca^{2+} entry through such channels is the only Ca^{2+} source related to spontaneous release of presynaptic transmitter in this region. None of our manipulations aimed at blocking external Ca^{2+} entry into neural cells of the slice completely abolished mEPSCs, although in some instances their occurrence was severely depressed. Low-threshold Ca^{2+} channels are particularly notable in rat DRG neurons of medium to small diameter (Scroggs and Fox, 1992). The central fibers of neurons of this size category of DRG neurons terminate largely in the superficial dorsal horn of the spinal cord (Light and Perl, 1979a,b). Neurons of rat and hamster laminae I and II behaved similarly in the augmentation of mEPSC frequency produced by increases in $[\text{K}^+]_o$ and in the reversal of the latter by mibefradil, indicating that these actions are not species-specific. Nonetheless, it must be kept in mind that neurons of this region receive inputs from other sources than the primary afferent fibers. It is probable that the miniature excitatory events we studied were generated from a mixed population of terminals, only some of which stemmed from dorsal root fibers.

Observations that synaptic excitation generated by impulses in presynaptic terminals differs from those associated with the production of spontaneous miniature synaptic events at the same junctions have been reported previously in studies on the hippocampus (Cotman et al., 1986; Parfitt and Madison, 1993; Scharfman, 1993). We have put forth evidence for the novel idea that the voltage-sensitive channels for external Ca^{2+} entry important for mEPSC generation are of a low-threshold

type (i.e., similar to T-type). The spontaneous miniature events commonly seen at chemically mediated synaptic junctions by definition occur in the absence of incoming impulses over the presynaptic fibers. Therefore, it is not surprising that the release of transmitter from such terminals, while at rest, depends on the availability of Ca²⁺ entering through channels opening at close to resting membrane potentials. We argue that such a relationship has special importance in the spinal superficial dorsal horn because the frequency of excitatory miniature events in a number of its neurons is sufficiently high to have an impact on their excitability. Excitatory transients occurring as often as 10–30/sec, each lasting 10–30 msec, could easily coincide with evoked activity appearing in other terminals and summate to threshold levels for a postsynaptic neuron. This point is especially important in considering the functional attributes of a region receiving a primary afferent input directly related to pain and temperature sensations. It implies that miniature excitatory events can contribute to the background excitability of central neurons whose activity in normal and pathological circumstances are related to these experiences. Whether the relationship of low-threshold Ca²⁺ channels to mEPSC generation holds more broadly in the mammalian CNS is not established but appears to be a likely possibility.

REFERENCES

Bao J, Li J, Perl ER (1997) Different presynaptic Ca²⁺ channels influence evoked EPSCs and spontaneous, miniature EPSCs in rodent spinal I and II neurones. *J Physiol (Lond)* 504:P:175P.
 Bao J, Li J, Wall B, Perl ER (1995) Low threshold (T type) Ca²⁺ channels control miniature EPSC frequency in the spinal substantia gelatinosa. *Soc Neurosci Abstr* 21:1572.
 Bardoni R, Goldstein PA, Lee CJ, Gu JG, MacDermott AB (1997) ATP P_{2X} receptors mediate fast synaptic transmission in the dorsal horn of the rat spinal cord. *J Neurosci* 17:5297–5304.
 Bessou P, Perl ER (1969) Response of cutaneous sensory units with unmyelinated fibers to noxious stimuli. *J Neurophysiol* 32:1025–1043.
 Bezprozvanny I, Tsien RW (1995) Voltage-dependent blockade of diverse types of voltage-gated Ca²⁺ channels expressed in *Xenopus* oocytes by the Ca²⁺ channel antagonist mibefradil (Ro 40–5967). *Mol Pharmacol* 48:540–549.
 Blanton MG, LoTurco J, Kriegstein AR (1989) Whole-cell recording from neurons in slices of reptilian and mammalian cerebral cortex. *J Neurosci Methods* 30:203–210.
 Boyd IA, Martin AR (1956) The end-plate potential in mammalian muscle. *J Physiol (Lond)* 132:74–91.
 Byerly L, Hagiwara S (1988) Calcium channel diversity. In: Calcium and ion channel modulation (Grinnell AD, Armstrong D, Jackson MB, eds), pp 3–18, New York: Plenum.
 Cervero F, Iggo A (1980) The substantia gelatinosa of the spinal cord: a critical review. *Brain* 103:717–772.
 Christensen BN, Perl ER (1970) Spinal neurons specifically excited by noxious or thermal stimuli: marginal zone of the dorsal horn. *J Neurophysiol* 33:293–307.
 Cotman CW, Flatmen JA, Ganong AH, Perkins MN (1986) Effects of excitatory amino acid antagonists on evoked and spontaneous excitatory potentials in guinea-pig hippocampus. *J Physiol (Lond)* 378:403–415.
 del Castillo J, Katz BJ (1954) Quantal components of the end-plate potential. *J Physiol (Lond)* 124:560–573.
 Earle KM (1952) The tract of Lissauer and its possible relation to the pain pathway. *J Comp Neurol* 96:93–111.
 Evans RJ, Derkach V, Suprenant A (1992) ATP mediates fast synaptic transmission in mammalian neurons. *Nature* 357:503–505.
 Fatt P, Katz B (1952) Spontaneous subthreshold potentials at motor nerve endings. *J Physiol (Lond)* 117:109–128.
 Fox A, Nowycky MC, Tsien RW (1987) Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones. *J Physiol (Lond)* 394:149–172.

Fyffe REW, Perl ER (1984) Is ATP a central synaptic mediator for certain primary afferent fibers from mammalian skin? *Proc Natl Acad Sci USA* 81:6890–6893.
 Gottmann K, Pfrieger FW, Lux HD (1994) The formation of glutamatergic synapses in cultured central neurons: selective increase in miniature synaptic currents. *Dev Brain Res* 81:77–88.
 Hille B (1992) Ionic channels of excitable membranes, Ed 2, Sunderland, MA: Sinauer.
 Hubbard JI (1961) The effect of calcium and magnesium on the spontaneous release of transmitter from mammalian motor nerve endings. *J Physiol (Lond)* 159:507–517.
 Ikeda K, Saito Y, Nishiyama A, Takasaka T (1992) Na⁺-Ca²⁺ exchange in the isolated cochlear outer hair cells of the guinea-pig studied by fluorescence image microscopy. *Pflügers Arch* 420:493–499.
 Jahr CE, Jessell TM (1983) ATP excites a subpopulation of rat dorsal horn neurones. *Nature* 304:730–733.
 Kumazawa T, Perl ER (1978) Excitation of marginal and substantia gelatinosa neurons in the primate spinal cord: indications of their place in dorsal horn functional organization. *J Comp Neurol* 177:417–434.
 Larkman AU, Stratford K, Jack J (1991) Quantal analysis of excitatory synaptic action and depression in hippocampal slices. *Nature* 350:344–347.
 Li J, Perl ER (1994) Adenosine inhibition of synaptic transmission in the substantia gelatinosa. *J Neurophysiol* 72:1611–1621.
 Li J, Perl ER (1995) ATP modulation of synaptic transmission in the spinal substantia gelatinosa. *J Neurosci* 15:3357–3365.
 Light AR, Perl ER (1979a) Reexamination of the dorsal root projection to the spinal dorsal horn including observations on the differential termination of course and fine fibers. *J Comp Neurol* 186:117–132.
 Light AR, Perl ER (1979b) Spinal termination of functionally identified primary afferent neurons with slowly conducting myelinated fibers. *J Comp Neurol* 186:133–150.
 Light AR, Trevino DL, Perl ER (1979) Morphological features of functionally defined neurons in the marginal zone and substantia gelatinosa of the spinal dorsal horn. *J Comp Neurol* 186:151–171.
 Luebke JI, Dunlap K, Turner TJ (1993) Multiple calcium channel types control glutamatergic synaptic transmission in the hippocampus. *Neuron* 11:895–902.
 Mele L (1969) Inhibition and activation of calcium transport in mitochondria. Effect of lanthanides and local anesthetic drugs. *Biochem* 8:2481–2486.
 Mishra SK, Hermsmeyer K (1994) Selective inhibition of T-type Ca²⁺ channels by Ro 40–5967. *Circ Res* 75:144–148.
 Mlinar B, Enyeart JJ (1993) Block of current through T-type calcium channels by trivalent metal cations and nickel in neural rat and human cells. *J Physiol (Lond)* 469:639–652.
 Nicholls JG, Martin AR, Wallace BG (1992) From neuron to brain, Ed 3. Sunderland, MA: Sinauer.
 Parfitt KD, Madison DV (1993) Phorbol esters enhance synaptic transmission by a presynaptic, calcium-dependent mechanism in rat hippocampus. *J Physiol (Lond)* 471:245–268.
 Pearson AA (1952) Role of gelatinous substance of spinal cord in conduction of pain. *Arch Neurol Psych* 68:515–529.
 Perl ER (1984) Pain and nociception. In: Handbook of physiology: the nervous system III, pp 915–975. Baltimore: Williams & Wilkins.
 Ramon y Cajal S (1909) Histologie du système nerveux de l’homme et des vertébrés. Paris: Maloine.
 Randall AD (1995) On the blockade of neuronal Na⁺ and K⁺ conductances by the Ca²⁺ channel antagonist Ro 405967. *Physiol Rev* 485:P:49P–50P.
 Randall AD, Tsien RW (1997) Contrasting biophysical and pharmacological properties of T-type calcium channels. *Neuropharmacology* 36:879–893.
 Ranson SW (1913) The course within the spinal cord of the non-medullated fibers of the dorsal roots: a study of Lissauer’s tract in the cat. *J Comp Neurol* 23:259–281.
 Reichling DB, MacDermott AB (1991) Lanthanum actions on excitatory amino acid-gated currents and voltage-gated calcium currents in rat dorsal horn neurones. *J Physiol (Lond)* 441:199–218.
 Réthelyi M, Szentágothai J (1973) Distribution and connections of afferent fibres in the spinal cord. In: Handbook of sensory physiology: somatosensory system, Vol 2 (Iggo A, ed), pp 207–252. Berlin: Springer.
 Réthelyi M, Light AR, Perl ER (1989) Synaptic ultrastructure of func-

- tionally and morphologically characterized neurons of the superficial spinal dorsal horn. *J Neurosci* 9:1846–1863.
- Scharfman HE (1993) Characteristics of spontaneous and evoked EPSPs recorded from dentate spiny hilar cells in rat hippocampal slices. *J Neurophysiol* 70:742–757.
- Scharfman HE, Schwartzkroin PA (1988) Electrophysiology of morphologically identified mossy cells recorded in the dentate hilus of guinea pig hippocampal slices. *J Neurosci* 8:3812–3821.
- Scroggs RS, Fox AP (1992) Calcium current variation between acutely isolated adult rat dorsal root ganglion neurons of different size. *J Physiol (Lond)* 445:639–658.
- Tang C-M, Presser F, Morad M (1988) Amiloride selectively blocks the low threshold (*T*) calcium channel. *Science* 240:213–215.
- Wheeler DB, Randall A, Tsien RW (1994a) Roles of N-type and Q-type Ca^{2+} channels in supporting hippocampal synaptic transmission. *Science* 264:107–111.
- Wheeler DB, Sather WA, Randall A, Tsien RW (1994b) Distinctive properties of a neuronal calcium channel and its contribution to excitatory synaptic transmission in the central nervous system. In: *Molecular and cellular mechanisms of neurotransmitter release*, (Stjärne L, Greengard P, Gillner S, Hökfelt T, Ottoson D, eds) pp 155–171. New York: Raven.
- Wyllie DJA, Manabe T, Nicoll RA (1994) A rise in postsynaptic Ca^{2+} potentiates miniature excitatory postsynaptic currents and AMPA responses in hippocampal neurons. *Neuron* 12:127–138.
- Yoshimura M, Jessell TM (1990) Amino acid-mediated EPSCs at primary afferent synapses with substantia gelatinosa neurones in the rat spinal cord. *J Physiol (Lond)* 430:313–335.