GABA-Receptor–Independent Dorsal Root Afferents Depolarization in the Neonatal Rat Spinal Cord

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Kremer, E. and A. Lev-Tov. GABA-receptor–independent dorsal root afferents depolarization in the neonatal rat spinal cord. J. Neurophysiol. 79: 2581–2592, 1998. Dorsal root afferent depolarization and antidromic firing were studied in isolated spinal cords of neonatal rats. Spontaneous firing accompanied by occasional bursts could be recorded from most dorsal roots in the majority of the cords. The afferent bursts were enhanced after elevation of the extracellular potassium concentration ([K⁺]e) by 1–2 mM. More substantial afferent bursts were produced when the cords were isolated with intact brain stem. Rhythmic afferent bursts could be recorded from dorsal roots in some of the cords during motor rhythm induced by bath-applied serotonin and L-methyl-D-aspartate (NMDA). Bilaterally synchronous afferent bursts were produced in pairs of dorsal roots after replacing the NaCl in the perfusate with sodium-2-hydroxyethansulphonate or after application of the γ-aminobutyric acid-A (GABA_A) receptor antagonist bicuculline with or without serotonin (5-HT) and NMDA. Antidromic afferent bursts also could be elicited under these conditions by stimulation of adjacent dorsal roots, ventrolateral funiculus axons, or ventral white commissural (VWC) fibers. The antidromic bursts were superimposed on prolonged dorsal root potentials (DRPs) and accompanied by a prolonged increase in intraspinal afferent excitability. Surgical manipulations of the cord revealed that afferent firing in the presence of bicuculline persisted in the hemisected cord, and it was observed after removal of their ventral horns. Cutting the VWC throughout its length did not perturb the bilateral synchronicity of the discharge. These findings suggest that the activity of dorsal horn neurons is sufficient to produce the discharge and that the bilateral synchronicity can be maintained by cross connectivity that is relayed from side to side dorsal to the VWC. Antagonists of GABA_A, 5-HT/5-HT_1C, or glutamate metabotropic group II and III receptors could not abolish afferent depolarization in the presence of bicuculline. Depolarization comparable in amplitude to DRPs, could be produced in tetrodotoxin-treated cords by elevation of [K⁺]e to the levels reported to develop in the neonatal rat spinal cord in response to dorsal root stimulation. A mechanism involving potassium transients produced by neuronal activity therefore is suggested to be the major cause of the GABA-independent afferent depolarization reported in our study. Possible implications of potassium transients in the developing and the adult mammalian spinal cord are discussed.

INTRODUCTION

Primary afferent depolarization (PAD) in vertebrate spinal cords develops by activation of primary afferents of nonantagonistic muscles (Burke and Rudomin 1977; Rudomin 1990), by stimulation of descending pathways (reviewed by Rudomin 1994; Rudomin et al. 1993), and by the activity of the central pattern generators (CPGs) for locomotion (Dubuc et al. 1988; Duenas and Rudomin 1988; Gossard 1996; Gossard et al. 1991) and scratching (Baev et al. 1991; Bayev and Kostyuk 1981). The sensory-evoked PAD has been linked to presynaptic inhibition of Ia excitatory postsynaptic potentials that is produced by stimulation of the same pathway and that has a similar time course (Burke and Rudomin 1977; Jankowska 1992; Nicoll and Alger 1979; Rudomin 1990; Schmidt 1971). Both phenomena have been attributed to release of γ-aminobutyric acid (GABA) from axo-axonic synapses, and it has been indicated that PAD is mediated by GABA_A receptors (Curtis and Lodge 1982; Rudomin et al. 1981), whereas presynaptic inhibition is mediated by presynaptic GABA_B receptors (Burke and Rudomin 1977; Nicoll and Alger 1979; Rudomin et al. 1993) and GABA_A receptors (Rudomin et al. 1993; see also Edwards et al. 1989; Lev-Tov et al. 1988a for the cat; Konnerth et al. 1990; Lacey 1997; Lev-Tov and Pinco 1992; Wang and Dun 1990 for the neonatal rat; Peng and Frank 1989 for the frog spinal cord; Alford and Grillner 1991 for the lamprey). Studies of the frog (Sykova and Vyklicky 1978) and rat (Thompson and Wall 1996) spinal cord, however, demonstrated that a late phase of PAD persisted in the presence of the GABA_A antagonist picrotxin, so that generation of PAD might involve other transmitters or alternative non-synaptic mechanisms. Recent studies of PAD in the rat spinal cord revealed that GABA_B receptor antagonists had no effect on dorsal root potentials (DRPs) (Lacey 1997; Thompson and Wall 1996) and that the serotonin type 2 (5-HT_2)/5-HT_1C receptor antagonist methysergide attenuated DRPs (Thompson and Wall 1996). Other studies of the lamprey (Alford and Grillner 1991) and recent studies of the chick (N. Chub and M. J. O’Donovan, personal communication) spinal cord suggested an involvement of GABA_A receptors in PAD. The present work therefore was aimed at finding the extent of GABA_A-independent afferent depolarization in the isolated mammalian spinal cord and at assessing its possible underlying mechanisms. We report that prolonged DRPs accompanied by antidromic afferent discharge and by a prolonged increase in intraspinal afferent excitability appear after bath application of bicuculline or after removal of chloride from the bath. The afferent depolarization and firing persisted when the GABA_A and GABA_B receptors, which have been shown to mediate presynaptic inhibition, were blocked by their specific antagonists or when a mixture of antagonists of GABA_A, GABA_B, 5-HT_2/5-HT_1C, and the glutamate metabotropic group II and III presynaptic receptors was added to the bath. Possible mechanisms that may be involved in generation of afferent depolarization under these conditions are discussed. Some of our preliminary results appeared in an abstract (Kremer and Lev-Tov 1997b).

METHODS

Preparation

Experiments were performed on the en bloc spinal cord preparation (see Kudo and Yamada 1987; Smith and Feldman 1987) iso-
Stimulation and recordings

Pairs of lumbar dorsal and ventral roots (usually L2–L4) were placed in suction electrodes and prepared for high gain AC recordings at 0.1 Hz to 10 kHz or, when required, for DC recordings. An adjacent more caudal dorsal root (usually L3–L5) and the surgically peeled (see Pinco and Lev-Tov 1994) ventrolateral funiculus (at T8 level) were placed in suction electrodes and prepared for a constant current stimulation. The ventral white commissure (VWC) was stimulated by the use of a bipolar tungsten electrode (WPI Stereotrode, impedance = 0.45–0.55 MΩ).

Antidromic excitability testing (see Wall 1958)

The cord was impaled from its ventrolateral aspect by 0.6–1 MΩ platinum-iridium electrodes. The electrode was localized to the segmental motor column as indicated from the intraspinal field potential elicited by antidromic stimulation of the segmental ventral root or to the lamina VII region (depth ≥500 μM, no detectable field on antidromic stimulation of the segmental ventral root, substantial synaptic field on stimulation of the segmental dorsal root). Electrode positions then were adjusted in small steps to produce antidromic afferent responses in the segmental dorsal root on low-intensity intraspinal stimulation. The stimulation intensity was adjusted to produce submaximal responses. To confirm that the responses were elicited by direct afferent stimulation and not by synaptically mediated afferent depolarization, the preparation (in some experiments) was bathed first in normal and then in low calcium-high magnesium Krebs saline. Action potentials that were elicited under these conditions by intraspinal stimulation had similar shapes, amplitudes and latencies (see Fig. 5). During the course of the experiments, antidromic afferent responses with and without conditioning (stimulation of more caudal dorsal roots) were elicited by repetitive intraspinal stimulation at 1 Hz, during and in between activity bursts, recorded continuously by the use of a PCM recorder and stored for off-line analyses.

Pharmacological substances

A mixture of 5-HT (Sigma, 20–30 μM) and N-methyl-D-aspartate (NMDA, Sigma, 2 μM) was used to induce locomotor-like activity in the spinal cord. Strychnine and bicusculine methylxanthide (Sigma), and 2-hydroxyysaclofen (RBI) were used to block glycine, GABAA, and GABA B receptors, respectively. The 5-HT/5HT1C receptor antagonist methysergide maleate (RBI) was used to block the serotonin type 2 receptors that are associated with afferent depolarization. (S)-2-Amino-2-methyl-4-phosphonobutyric acid (MAPA, Tocris Cookson) and (2S,3S,4S)-2-methyl-2(carboxycyclopropyl)glycine (MCCG, Tocris Cookson) were used to block the metabolotropic 2-amino-4-phosphonobutyric acid (AP4) receptor (group III) and the metabotropic AP4 resistant (group II) glutamate receptors (see Jane et al. 1994). The sodium salt of 2-hydroxyethansulfonic acid was used as a chloride-free substitute for NaCl in the bathing media.

Data acquisition and analysis

Data (wideband, either 0.1 Hz to 10 kHz or DC recordings) were recorded continuously using a high-speed (22–88 kHz) PCM recorder (Neurodata). Data then were digitized (Digidata 1200, Axon Instruments) filtered using high- and low-pass filters (10 Hz to 10 kHz for discharge, DC: 200 Hz or AC: 0.1–200 Hz for DRPs) and stored for subsequent off-line computer analyses.

RESULTS

Antidromic afferent discharge in the neonatal rat spinal cord

Figure 1A shows recordings from ventral (top) and dorsal (bottom) roots in the isolated cord. The activity pattern of dorsal root afferents under these conditions was irregular and composed of low-frequency short bursts. Spontaneous antidromic activity with a similar pattern was found in 9/12 experiments performed in this series. Figure 1B shows that stimulation of a more caudal dorsal root produced short-duration antidromic firing in the recorded dorsal root afferents and orthodromic reflex in the respective ventral root. Enhanced antidromic afferent bursts were found in 3/3 spinal cord preparations that were isolated with an intact brain stem (Fig. 1C). Brain stem-Spinal cord [also Vinay et al. 1997], and in 5/5 preparations bathed in media in which [K+] e was elevated from 4 to 5–6 mM (Fig. 1C, 6 mM K+).

Bath application of 5-HT and NMDA to isolated rat spinal cords has been reported to induce an alternating left-right rhythmic activity resembling the locomotor rhythm in pairs of ventral roots (Cowley and Schmidt 1997; Kjaerulf and Kiehn 1996; Kremer and Lev-Tov 1997a; Sqalli Houssaini et al. 1993). Recordings from the ventral root of L3 and dorsal root of L4 in the presence of 20 μM 5-HT and 2 μM NMDA are shown in Fig. 1C (5-HT, NMDA). Short rhythmic afferent discharge was in phase with motor axons discharge could be seen clearly under these conditions. The rhythmic discharge of dorsal root afferents in the presence of 5-HT and NMDA was found in only in 2/7 of the preparations used in this series of experiments (possibly due to the depressive effect of serotonin on spontaneous DRPs) (Lopes-Garcia and King 1996).

Antidromic afferent firing in the presence of bicuculline or/and in the absence of [Cl−] e

GENERAL PHENOMENOLOGY. Because dorsal root firing has long been attributed to GABA A-receptor–mediated PAD, the experiments described above were repeated in the presence of the GABA A-receptor antagonist bicuculline. Figure 2A shows recordings from the left and right ventral and dorsal roots of L3 after addition of 20 μM bicuculline to a preparation in the presence of 5-HT and NMDA (Fig. 2A, NMDA, 5-HT, Bicuculline). A bilaterally synchronous dorsal root afferent discharge appeared shortly (2–4 min) after addition of bicuculline. Addition of bicuculline to another preparation in the absence of 5-HT and NMDA (Fig. 2A, NMDA, 5-HT, Bicuculline) also revealed a substantial dorsal root afferents bursts. Similar results were obtained in each of the 24 experiments performed in this series. Bilateral ventral dorsal root bursts also could be induced by replacing the NaCl in the Krebs saline by the sodium salt of 2-hydroxyethansulfonic acid (thereby removing 96% of the extracellular chloride ([Cl−] e from the bathing medium) in the presence of 5-HT and NMDA (Fig. 2B, NMDA, 5-HT, Cl− free). The strong bilateral discharge of ventral and dorsal roots per-
FIG. 1. Antidromic afferent firing in the spinal cord. A: spontaneous ventral root efferent and antidromic dorsal root afferent firing recorded from the left L2 ventral (L-VL2) and dorsal (L-DL2) roots respectively, in normal Krebs saline. B: recordings of ventral root and of antidromic dorsal root afferent firing elicited in the left ventral and dorsal roots of L2 by single pulse stimulation (30 μA, 100 μs, −) of the left L3 dorsal root. C: recordings of ventral root efferent and dorsal root afferent bursts from the left L3 segment in a spinal cord preparation that was isolated with an intact brain stem (cut at the pontomedul- lary junction, Brain stem-Spinal cord), in a spinal cord preparation that was bathed in 6 mM K⁺ Krebs saline (6 mM K⁺), and recordings from the right L4 dorsal and the right L3 ventral root in another preparation in the presence of 20 μM serotonin (5-HT) and 2 μM N-methyl-D-aspartate (NMDA; 5-HT, NMDA).

Antidromic afferent discharge in the presence of bicuculline also could be evoked by electrical stimulation. Figure 2C shows that antidromic afferent bursts were evoked in the left and right L3 dorsal roots by low-intensity (12 μA) single pulse stimulation of the left L4 dorsal root (Fig. 2C, DR Stim.), and by short stimulus trains (5–10 pulse, 40 Hz) applied to the ventral white commissure at L3 level (Fig. 2C, VWC Stim) or to the ventrolateral funiculus at T8 level (VLF Stim.).

At the next stage of the project, we used surgical manipulation to clarify the basis for the bilateral synchronicity of antidromic afferent discharge in the presence of bicuculline and to localize the regions of the cords that are essential for generation of the discharge.
LOCALIZATION OF THE FIBERS MEDIATING THE BILATERAL SYNCHRONICITY OF AFFERENT DISCHARGE. Recent studies of the locomotor rhythm in the neonatal rat spinal cord revealed that the connectivity required to maintain left-right hindlimb alternation is relayed from side to side through the VWC (Kjaerulff, and Kiehn 1996). To determine whether the VWC is used also to relay the fibers that are involved in bilateral synchronization of the antidromic afferent bursts in the presence of bicuculline, we tested the effects of a complete midsagittal lesion of the VWC on the synchronicity of the bursts.

A  NMADA, 5HT, Bicuculline (E-169)
L-VL3  
R-VL3  
L-DL3  
R-DL3

B  NMADA, 5HT, Cl-free (E-190)
L-VL3  
R-VL3  
L-DL3  
R-DL3

C  DR-Stim. (E-169)
L-VL3  
R-VL3  
L-DL3  
R-DL3

VWC Stim. (E-173)
L-VL3  
R-VL3  
L-DL3  
R-DL3

VLF (R-T8) Stim. (E-206)
L-VL2  
R-VL2  
L-DL2  
R-DL2

10 s  
5 s
Figure 3 shows a left-right alternating rhythm recorded from the ventral roots of L3 (Control, top two traces). The concurrently recorded dorsal roots did not show in this case, clear rhythmic activity. Addition of 20 μM bicuculline induced bilateral synchronization of both the ventral and dorsal root activity (Fig. 3, Bicuculline). Washout of the bicuculline restored the left-right alternating activity (Fig. 3, Bicuculline Wash). Longitudinal destruction of the VWC by a fine tungsten wire, perturbed, as expected, the left-right alternation, leading to left-right independent rhythms (VWC-Cut). Addition of bicuculline under these conditions, induced bilaterally synchronous ventral and dorsal root discharges (Bicuculline VWC-Cut). These findings indicated that the bilateral synchronization of antidromic afferent discharge in the presence of bicuculline and the bicuculline-induced cross-excitation of motoneurons do not depend on an intact VWC and can be obtained by fibers that cross the cord dorsal to the VWC. Similar results were obtained in eight additional spinal cord preparations in which the VWC was midsagittally cut.

REGIONAL SOURCE OF ANTIDROMIC AFFERENT DISCHARGE. Figure 4A shows the results obtained in one of the five experiments in which we tested whether a bilaterally intact
cord is required for generation of the afferent discharge in the presence of bicuculline. The “spontaneous” and dorsal root-evoked antidromic afferent firing that was observed in the presence of bicuculline, persisted in the midsagittally split cord. Similar antidromic activity was observed in each of the five cords that were tested in this series. In three of these preparations, the ventral horn was removed from the hemicord and the afferent activity was recorded from the segmental dorsal roots before and after stimulation of a more caudal dorsal root. Figure 4B shows that “spontaneous” and dorsal root-evoked afferent discharge persisted in the surgically detached dorsal horn. These findings indicated that the activity of the disinhibited dorsal horn neurons in the presence of bicuculline was sufficient enough to produce antidromic afferent firing.

Dorsal root afferent depolarization in the presence of bicuculline

Antidromic dorsal root afferent firing in mammals is attributed to synaptically induced PAD (reviewed in: Burke and Rudomin 1977; Kerkut and Bagust 1995; Schmidt 1971). PAD can be recorded directly from intraspinal afferents (see Burke and Rudomin 1977; Schmidt 1971 for reviews; see also Lev-Tov et al. 1983, 1988b) or inferred from changes in afferent excitability (Wall 1958) or from recordings of slow DRPs (Barron and Matthews 1938; see Burke and Rudomin 1977; Schmidt 1971 for reviews). Because of the small diameter of dorsal root afferents in the newborn rats, we have used the two latter methods to reveal whether a significant afferent depolarization persisted after blocking the GABA_A receptors with bicuculline.

Excitability testing of intraspinal afferents in the presence of bicuculline. Figure 5A shows a schematic view of the preparation used for excitability testing. Generally speaking, after positioning the stimulation electrode in the ventrolateral or the intermediate gray region (see Methods), antidromic action potentials could be elicited in the segmental dorsal root by low-intensity (5–16 μA, 100 μs) intraspinal stimulation, before and after conditioning stimulation of an adjacent (usually more caudal) dorsal root. The antidromic action potentials shown in Fig. 5A, inset, were elicited by intraspinal stimulus pulses and sampled as the preparation was bathed first in normal (2 mM Ca^{2+}, 1 mM Mg^{2+}, Fig. 5A1), and then in 0.1 mM Ca^{2+} and 5 mM Mg^{2+} (2) Krebs saline (in which the synaptic activity is abolished). The similar latencies, amplitudes and waveforms of these records indicated that the afferent action potentials were elicited by a direct antidromic activation of the intraspinal afferents. Figure 5B shows recordings from the left
FIG. 5. Excitability testing of intraspinal afferents in the presence of bicuculline. A: schematic view of the preparation (ventral side up) and the arrangements used for stimulation and recordings. Suction electrode recordings are obtained from the segmental dorsal and ventral roots (DR and VR Recordings). Antidromic testing responses (DR recordings) are elicited by stimulation electrode (Intraspinal Stim.) that is positioned to the gray matter of the ventrolateral cord or to the intermediate gray (see METHODS). Conditioning stimulation is applied to a caudal dorsal root by suction electrode (DR-Stim.). The computer averaged antidromic testing responses (20-sweep each, inset) were obtained in normal (2 mM Ca²⁺, 1 mM Mg²⁺, 1) and in low-calcium, high-magnesium (0.1 mM Ca²⁺, 5 mM Mg²⁺, 2) Krebs saline, on 5-µA intraspinal stimulus pulses applied at 1 Hz. B: recordings from the left dorsal and ventral roots of L3 (top and bottom, respectively) during repetitive intraspinal stimulation at 1 Hz. Conditioning stimuli (15-µA square pulses) were applied to the L4 dorsal root at 40- to 50-s intervals (r). Normalized peak-to-peak amplitude of the antidromic action potentials (normalized by the mean control values, bars) is plotted as a function of time (graph). Superimposed records are computer averaged antidromic responses (5-sweep each) sampled before and after the respective conditioning stimuli.

dorsal (top) and ventral (bottom) roots of L3 during repetitive (1 Hz) intraspinal stimulation. To elicit antidromic discharge at a desired time, conditioning stimuli were applied to the L4 dorsal root every 40–50 s (→). The conditioning stimulation induced a prolonged increase in the amplitude of the antidromic action potentials (see the computer averaged action potentials recorded before and immediately after 2 of the conditioning stimuli). The peak-to-peak amplitude of the antidromic action potentials obtained during the epoch that is shown in Fig. 5B was measured, normalized by a mean control value that has been reached 20–30 s after a conditioning stimulus (bars), and plotted as a function of time (B, graph). It can be seen clearly that a single conditioning stimulus was capable of inducing an increase in intraspinal afferent excitability lasting ≈20 s long. The experiment described above was repeated in 12 additional preparations. The intraspinal antidromic afferent excitability in these preparations was tested in 83 laminae IX and VII locations. A substantial and prolonged (1–20 s) increase in antidromic afferent excitability on conditioning stimulation of nonsegmental dorsal roots was found in the presence of 20 µM bicuculline in 47 of the 71 (66%) lamina IX and in 11 of the 12 (92%) lamina VII locations. A substantial increase in antidromic afferent excitability also was found under these conditions during the spontaneously occurring antidromic burst activity (not shown).

DRPs IN THE PRESENCE OF BICUCULLINE. DRPs are used as an index for afferent depolarization. To confirm that DRPs persist in the presence of GABA A receptor blockers in the neonatal rat spinal cord (e.g., Sykova and Vyklícky 1978).
for the frog; Thompson and Wall 1996 for the adult rat spinal cord), we recorded spontaneous and dorsal root-evoked DRPs (DR-DRPs) before and after addition of bicuculline. Figure 6 describes an experiment in which the left dorsal root of L4 was stimulated, and the responses elicited in the left L3 ventral and dorsal roots were recorded before (Control) and after addition of 20 μM bicuculline (Bicuculline). During the control period, the stimulation elicited few action potentials in the recorded L3 ventral and dorsal roots. The simultaneously recorded L3-DRPs (L3 DRPs Control), reached ~500 μV and lasted ~600 ms. After addition of bicuculline, the same stimulus elicited prolonged bursts of action potentials in the recorded dorsal and roots (Bicuculline). The L3 DRPs under these conditions (L3-DRPs, Bicuculline) had a delayed onset, a slower rise time (~180 ms from 20 to 80% peak), a suppressed amplitude (250 μV), and a prolonged (>2,500 ms) duration. Analyses of the six experiments performed in this series, revealed that the amplitude of DRPs declined to 0.54 ± 0.24 (mean ± SD) of their control value, whereas their duration was increased by a factor of 2.32 ± 0.17 (n = 6) after addition of 20 μM bicuculline to the bath. At the next stage of the study, we further characterized the pharmacology of dorsal root afferent depolarization in the presence of bicuculline.

**Pharmacology of the dorsal root afferent depolarization in the presence of bicuculline**

Because GABA is known to activate not only GABA_A but also GABA_B receptors and because recent studies revealed that 5-HT_2 presynaptic receptors (Thompson and Wall 1996) contributed to DR-DRPs in the adult rat spinal cord, we tested whether these receptors were involved in generation of DRPs in the presence of bicuculline. Preparations were bathed in Krebs saline that contained 20 μM bicuculline. DRPs and dorsal and ventral root firing were recorded before and after the addition of the GABA_A receptor blocker 2-hydroxysaclofen and the 5-HT_2/5-HT_1C receptors blocker methysergide. The results obtained in one of these experiments are shown in Fig. 7A. Stimulation of the left L3 dorsal root in the presence of bicuculline produced ventral and dorsal root firing and a 1.5-s–long DRP (Fig. 7A).

After addition of 2-hydroxysaclofen (200 μM), stimulation of the L3 dorsal root induced much longer ventral root firing accompanied by ~3-s–long, slowly rising DRPs (Fig. 7A2), and an extended duration of afferent discharge (as compared with the discharge in the presence of bicuculline alone). Addition of methysergide (0.5 μM) to the bathing medium changed the pattern of the firing elicited in the L2 ventral and dorsal roots on stimulation of the L3 dorsal root but did not affect the duration nor the amplitude of the DRPs (Fig. 7A3). The results obtained in three additional experiments showed that the duration of DRPs produced in the presence of bicuculline (2.55 ± 0.4 s, n = 4, 10-sweep computer averages were sampled in each experiment) was prolonged by 8–92% (45 ± 31%, n = 4) after addition of 5-hydroxysaclofen and methysergide to the bath.

To test whether the afferent depolarization and antidromic firing in the presence of bicuculline involved activation of presynaptic glutamate receptors (see Scanziani et al. 1997), we recorded spontaneous and DR-evoked ventral and dorsal root firing in the presence of bicuculline, and then tested the effects of the group II and group III metabotropic glutamate receptor antagonists, MCCG and MAP4 (Jane et al. 1994), in three different preparations. Figure 7B shows that stimulation of the right L5 dorsal root in the presence of bicuculline and strychnine induced a bilaterally synchronous ventral root firing (recorded from the left and right L2 ventral roots) and antidromic discharge in the right and left dorsal roots of L4 (Bicuculline, Strychnine). The ventral root and the antidromic afferent firing persisted after addition of 100 μM MAP4 and 200 μM MCCG to the bath (Bicuculline, Strychnine, MAP4, MCCG). In three additional experiments, we found that a substantial bilaterally synchronous antidromic afferent firing could be elicited in the segmental dorsal root on stimulation of a more caudal dorsal root in the presence of a mixture of bicuculline (20 μM), strychnine (2 μM), 2-hydroxysaclofen (200 μM), methysergide (0.5–1 μM), MAP4 (100 μM), and MCCG (200 μM). The results of one of these experiments are shown in Fig. 7C.

Finally, because afferent depolarization persisted in the presence of all the receptor antagonists described above, we tested whether elevation of [K⁺]e to the levels that have...
FIG. 7. Pharmacology of the dorsal root afferent depolarization. A: simultaneous recordings of the left L2 ventral and dorsal root firing, and of computer-averaged L2-DRPs (10 sweep each) elicited by stimulation of the left L3 dorsal root in the presence of 20 μM bicuculline (1), after addition of 200 μM 2-hydroxysaclofen (2), and after application of 0.5 μM methysergide (3) to the bath. Time calibration of these DRPs applies also to the top 2 traces. Averaged L2-DRPs are also shown superimposed with an expanded time scale (1–3). B: ventral (L2) and antidromic dorsal root afferent (L4) firing elicited by stimulation of the right L5 dorsal root are shown in the presence of a mixture (see text). S, spontaneous burst. Stimulation intervals in A–C were 30, 20, and ≥35 s, respectively. D: effect of elevated potassium concentration on dorsal root depolarization in the absence of neuronal activity. Continuous DC recordings from the left L3 dorsal root in a preparation treated with 5 μM TTX are shown before, during (between the 2 arrows), and after perfusion of the preparation with Krebs saline in which the concentration of KCl was elevated from 4 to 10 mM.
been reported to develop in the neonatal rat spinal cord during dorsal root stimulation (10–10.5 mM) (see Jen-de-lova and Sykova 1991; Walton and Chesler 1988) is capable of producing a detectable dorsal root depolarization. The effects of elevated [K⁺]e were tested in preparations in which the neuronal activity was blocked by bath-applied tetrodotoxin (TTX; 5 μM). Recordings were obtained first from spinal cords with intact dorsal roots to reveal the maximal DC shift produced by exposure to Krebs saline with 10 mM KCl (Fig. 7D). Recordings then were obtained from dorsal roots that were disconnected from the cord at the entry zone to reveal the relative contribution of the extra-and intraspinal part of the afferents to the depolarization induced by the elevated potassium. Finally, recordings were obtained from the experimental chamber in the absence of preparation to reveal the extent of DC shifts produced by exposure of the Ag/AgCl electrodes to the 10 mM KCl Krebs saline. Our results revealed that exposure to Krebs saline with 10 mM KCl produced 1,290 ± 50 μV (n = 4) in the intact preparation, 470 ± 70 μV (n = 4) in the isolated dorsal root, and 160 ± 30 μV (n = 4) in the absence of a preparation. Thus the intraspinal part of the afferent contributed 820 μV, whereas the extraspinal part of the afferent contributed 310 μV to the overall DC shift during exposures to 10 mM KCl Krebs saline.

**DISCUSSION**

PAD accompanied by antidromic afferent firing has been reported to occur in a variety of mammalian spinal cord preparations (for review, see Kerkut and Bagust 1995). The sensory-evoked PAD has been shown to be mediated by activation of presynaptic GABA₅ receptors, and it has been suggested to contribute to the underlying mechanism of presynaptic inhibition (see Graham and Redman 1994; Schmidt 1971), although a conductance dependent shunting of presynaptic action potential is sufficient enough to account for presynaptic inhibition (see Segev 1990). In the present study, the GABA₅-mediated inhibition and afferent depolarization were blocked by bathing the preparation in chloride free Krebs saline or by application of the specific GABA₅ receptor blocker bicuculline. Under these conditions we observed prolonged DRPs, long-term increase in intraspinal afferent excitability, and prolonged bilaterally synchronous antidromic afferent bursts.

A number of mechanisms might account for the GABA₅-independent afferent depolarization in the neonatal rat spinal cord. The first is activation of a different type or class of presynaptic receptors. GABA in the vertebral spinal cord, has been shown to activate not only GABA₅, but also pre-and postsynaptic GABA₉ receptors (see Bormann 1988; Bowery 1993; Malcangio and Bowery 1996; Mott and Lewis 1994). Studies of the lamprey spinal cord (Alford and Grillner 1991) and of the embryonic chick spinal cord (N. Chub and M. J. O’Donovan, personal communication), suggested that GABA₉ receptors may be capable of contributing to PAD. Studies of the adult (Thompson and Wall 1996) and neonatal (Lacey 1997) rat spinal cord, however, showed that the GABA₉ antagonists CGP 36742 and CGP56999A failed to reduce DRPs. In Fig. 7, we showed that the DRPs and antidromic afferent firing elicited in the presence of bicuculline could not be blocked and actually were prolonged after application of the GABA₉ receptor blocker 2-hydroxysaclofen. Figure 7 also showed that blocking of the 5-HT₂ receptor, something which recently has been suggested to contribute to DRPs in the adult rat spinal cord (Thompson and Wall 1996), did not abolish the prolonged DRPs nor the antidromic afferent discharge that was induced in the presence of bicuculline and 2-hydroxysaclofen. Thus the involvement of an axo-axonally released transmitter in the mechanisms underlying the afferent depolarization described above is probably unlikely. Another transmitter-mediated mechanism that should be considered stems from the recent findings that the glutamate released during massive activation of mossy fibers in the hippocampus has been suggested to diffuse and activate nearby glutamate metabotropic receptors (Scanziani et al. 1997). Contribution of similar mechanisms to the bicuculline-resistant afferent depolarization is not consistent with our findings that the spontaneous and the DR-evoked antidromic firing elicited in the presence of bicuculline could not be abolished by a mixture of the specific antagonists of the presynaptic glutamate metabotropic receptors, MAP4 and MCCG (Fig. 7), either with or without 2-hydroxysaclofen and methysergide. Thus although transmitter mediated mechanisms cannot be completely excluded, they do not seem to be the preferable explanation for the GABA-receptor–independent afferent depolarization. This brings us to an alternative mechanism.

Neuronal activity produces potassium transients in the vicinity of active neurons. These transients depend on the duration and frequency of neuronal firing, on the synchronicity of their activation, and on the extracellular space and the efficacy of the processes that are responsible for potassium clearance. The intense neuronal discharge that is characteristic of various pathological conditions therefore is followed by substantial potassium transients (for reviews, see Jefferys 1995; Sykova 1997). The massive discharge that accompanies disinhibition of neuronal networks in the presence of the GABA₅-receptor blocker picrotoxin also has been reported to induce potassium transients in the frog (Sykova and Vyklicky 1978) and the neonatal rat (Sykova et al. 1992) spinal cord. It therefore seems that potassium transients may be responsible for a major part of afferent depolarization in the presence of bicuculline. Our findings that 1) the bicuculline-resistant afferent depolarization could be produced in the surgically detached dorsal horn and at the intermediate gray regions that have been reported to yield the largest DR-evoked potassium transients in the neonatal rat (Walton and Chesler 1988); 2) that the duration of DRPs and antidromic firing varied with the degree of disinhibition of the network (the addition of 2-hydroxysaclofen and methysergide enhanced the disinhibition that has been induced by bicuculline and thereby prolonged the neuronal bursts and increased the duration of the concurrently recorded DRPs); and 3) that the afferent depolarization could be evoked in any spinal segment by stimulation of either dorsal root afferents at any segmental level or descending reticulospinal and ascending and descending propriospinal fibers traveling in the VLF as well as by stimulation of commissural fibers at different segmental levels, are all accordant with the notion that the GABA-independent afferent depolarization described earlier might be mediated by activity-dependent potassium transients.
The question arises whether these potassium transients are simply artifacts of the intense discharge that accompanies disinhibition of spinal networks by bicuculline, 2-hydroxy-6-salicylic acid, and methysergide. Studies of potassium transients in the isolated spinal cord of the neonatal rat revealed that single-pulse stimulation of dorsal root afferents increased the [K+]e by $\leq 2.5$ mM (Jendelova and Sykova 1991) and that afferent stimulation even at moderate frequencies (10 Hz) increased the [K+]e by $\leq 6.5$ mM (Jendelova and Sykova 1991; Walen and Chesler 1988). In the present study, we showed that similar increase in [K+]e (6 mM) produced depolarization of intraspinal afferents that was comparable in amplitude with DRPs in untreated preparations. Thus the potassium transients that were reported to occur in the neonatal rat spinal cord may contribute to the sensory-evoked PAD not only in the presence of bicuculline but also under normal conditions. Moreover, studies of the adult cat spinal cord revealed that repetitive activation of cutaneous afferents and of mixed afferents induced potassium transients that were sufficient to depolarize group Ia afferents (Jimenez et al. 1984). Hence, the effects of potassium transients are not restricted to the developing spinal cord. Another example in which potassium transients should be considered is the PAD and antidromic afferent discharge observed during fictive locomotion in the cat spinal cord (Dubuc et al. 1988; Duenas and Rudomin 1988; Gossard 1996; Gossard et al. 1991) and during neurochemically induced locomotion in the neonatal rat (Fig. 1, in the present study). Unlike the sensory-evoked PAD, the CPG-related PAD in the cat spinal cord was not associated with presynaptic inhibition of Ia EPSPs (Gossard 1996). It has been suggested therefore that this CPG-related PAD was not produced by presynaptic release of GABA and that it is likely to involve potassium transients (Gossard 1996). This notion is supported by the findings that phasic changes in [K+]e have been reported to accompany similar rhythmic behaviors such as fictive swimming in the lamprey spinal cord (Walen et al. 1984) and the inspiratory discharge in the ventral medulla of the neonatal rat (Brockhaus et al. 1993). In summary, we suggest that high-frequency synchronous activation of spinal neurons, not only during disinhibition or pathological conditions, but also during locomotion, swimming, scratching, respiration, and various reflexes, are capable of producing potassium transients that are sufficient (by themselves or by assisting transmitter-mediated mechanisms) to depolarize intraspinal afferents. These depolarized afferents may affect the orthodromic information conveyed by them not only in the presence of bicuculline but also under normal conditions. Moreover, studies of the adult cat spinal cord revealed that repetitive activation of cutaneous afferents and of mixed afferents induced potassium transients that were sufficient to depolarize group Ia afferents (Jimenez et al. 1984). Hence, group Ia spinal afferent terminations by GABA.

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