Dorsal Column Nuclei Neurons Recorded in a Brain Stem–Spinal Cord Preparation: Characteristics and Their Responses to Dorsal Root Stimulation

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Deuchars, Susan A., T. Trippenbach, and K. Michael Spyer. Dorsal column nuclei neurons recorded in a brain stem–spinal cord preparation: characteristics and their responses to dorsal root stimulation. J Neurophysiol 84: 1361–1368, 2000. Recordings were obtained from dorsal column nuclei (DCN) neurons in a neonatal rat brain stem–spinal cord preparation to study their basic electrophysiological properties and responses to stimulation of a dorsal root. Whole-cell patch-clamp recordings were made from 21 neurons that responded to dorsal root stimulation with a fast excitatory postsynaptic potential (EPSP). These neurons were located lateral to, but at the level of, the area postrema at depths of 100–268 μm below the dorsal surface of the brain. The neurons could be divided into groups according to the shape of their action potentials or voltage responses to hyperpolarizing current steps; however, the response profiles of the groups of neurons to dorsal root stimulation were not significantly different and all neurons were considered together. Dorsal root stimulation elicited excitatory postsynaptic potentials (EPSPs) in all neurons with a very low variability in onset latency and an ability to follow 100-Hz stimulation, indicating that they were mediated by activation of a monosynaptic pathway. The peak amplitude of the EPSP increased with membrane hyperpolarization, and applications of the non-NMDA receptor antagonists 6-nitro-7-sulfamoylbenzof[l]isquinoxaline-2,3-dione (NBQX) and 6,7-dinitroquinoxaline-2,3-dione (DNQX) decreased the amplitude of the EPSP to 14.2% of the control response (n = 6). The descending phase of the EPSP decreased with membrane hyperpolarization and was reduced by the N-methyl-D-aspartate (NMDA) receptor antagonist AP-5 (n = 2). The EPSPs were also reduced in amplitude by applications of the γ-aminobutyric acid-B (GABA_B) receptor agonist baclofen, which had no effect on membrane potential or input resistance. These results show that fast EPSPs in DCN neurons elicited by dorsal root stimulation are mediated by an excitatory amino acid acting at both non-NMDA and, to a lesser extent, NMDA receptors. In addition, GABA acting at presynaptic GABA_B receptors can inhibit these responses.

INTRODUCTION

The dorsal column nuclei (the gracile and cuneate nuclei) are known to be major relays in the transmission and processing of information from low-threshold mechanoreceptors. Anterograde labeling studies have shown that primary afferent inputs from the upper thoracic and cervical levels of the rat spinal cord ascend to the dorsal column nuclei and terminate in the cuneate and external cuneate nucleus (Basbaum and Hand 1973; Beck 1981). The primary afferent input to dorsal column nuclei (DCN) neurons may use glutamate as the main neurotransmitter since ionophoretic applications of glutamate in the vicinity of these neurons caused excitation (Galindo et al. 1967), while 1-hydroxy-3-aminopyrrolid-2-one (HA-966, an excitatory amino acid receptor antagonist) blocked excitatory synaptic transmission within the cuneate nucleus (Davies and Watkins 1973). In addition, glutamate immunoreactivity has been demonstrated in identified primary afferent terminals in the cuneate nucleus (De Biasi et al. 1994), while AMPA receptors are expressed by all neurons in the DCN (Propratillo et al. 1997). However, the exact nature of the fast response evoked by dorsal root stimulation and the effect of selective excitatory amino acid receptor antagonists has yet to be determined. This was the first aim of the study.

Inhibition of evoked activity in DCN neurons has been reported by many groups (see Willis and Coggeshall 1991) and this may involve both a post- and a presynaptic GABAergic input onto these neurons (Lue et al. 1996). The postsynaptic effect is mostly of short duration, while the presynaptic effect may be important in the more prolonged inhibition observed in the DCN (Anderson et al. 1964, 1970; Jabbur and Banna 1968, 1970). Various studies have investigated the role of γ-amino butyric acid-A (GABA_A) -mediated primary afferent depolarization in the presynaptic inhibition of DCN neurons (see Willis and Coggeshall 1991). However, it is likely that activation of presynaptic γ-aminobutyric acid-B (GABA_B) receptors which decrease synaptic activity may be the important mechanism involved in the prolonged inhibition observed in the dorsal column nuclei. Indeed, Newberry and Simmonds (1984) revealed a bicuculline resistant component of the negative wave of the field potential recorded in response to dorsal column stimulation in the gracile nuclei. Therefore, the second aim of this study was to explore the role of the GABA_B receptor in the DCN.

We have developed a neonatal rat brain stem–spinal cord preparation for recording from sympathetic preganglionic neurons (SPNs) to study the pharmacology of inputs onto these neurons (Deuchars et al. 1995a,b). The preparation is highly suitable for the study of dorsal root inputs onto neurons within the dorsal column nuclei since this long pathway is also main-
tained and dorsal roots can be stimulated to determine the responses of individual DCN neurons. Using whole-cell patch-clamp recordings from DCN neurons, the changes in membrane potential of these neurons evoked by stimulation of the dorsal roots can be determined and the effects of drugs on these responses can be resolved. This study examined 1) the basic membrane properties of neurons within the cuneate nucleus with an aim to grouping the neurons according to these characteristics, 2) their responses to dorsal root stimulation and the effects of excitatory amino acid antagonists, and 3) the effects of applications of baclofen and the selective GABA_B antagonist CGP35348 on the evoked responses to dorsal root stimulation to determine whether there is a presynaptic GABA_B receptor-mediated inhibition of these responses. These results have been presented in abstract form (Deuchars et al. 1997).

METHODS

Neonatal rats (2–5 days) were anesthetized initially with isoflurane. They were then placed on ice to maintain anesthesia by hypothermia as described previously (Deuchars et al. 1995a). A high decerebration was performed rapidly and the brain stem and spinal cord were isolated in artificial cerebrospinal fluid (aCSF) equilibrated with 95% O_2-5% CO_2 (see Deuchars et al. 1995b). The brain stem–spinal cords were then pinned onto Sylguard in a recording chamber with the dorsal surface of the brain stem uppermost. The spinal cords were then pinned onto Sylguard in a recording chamber with the dorsal surface of the brain stem uppermost. The spinal cords were twisted at the upper cervical level to allow simultaneous access to dorsal and ventral roots from the lower cervical levels. The preparation was rapidly and the brain stem and spinal cord were isolated in artificial cerebrospinal fluid (aCSF) equilibrated with 95% O_2-5% CO_2 (see Deuchars et al. 1995b). A high decerebration was performed rapidly and the brain stem and spinal cord were isolated in artificial cerebrospinal fluid (aCSF) equilibrated with 95% O_2-5% CO_2 (see Deuchars et al. 1995b). The brain stem–spinal cords were then pinned onto Sylguard in a recording chamber with the dorsal surface of the brain stem uppermost. The spinal cords were twisted at the upper cervical level to allow simultaneous access to dorsal and ventral roots from the lower cervical levels. The preparations were superfused at a rate of 5 ml/min with aCSF composed of (in mM) 128 NaCl; 3 KCl; 0.5 NaH_2PO_4 · H_2O; 1.5 CaCl_2 · 2H_2O; 1 MgSO_4; 23.5 NaHCO_3; 30 glucose, and 2 mannitol equilibrated with 95% O_2-5% CO_2 and maintained at 26–27°C. The dura mater was removed to increase the diffusion of O_2 to the tissue. Suction electrodes were advanced into the brain stem in 1.2-μm steps until a change in resistance was observed. Gentle suction was applied until a high-resistance (1–5 GΩ) seal was made; then, brief pulses of negative pressure caused rupture of the membrane. The electrodes were filled with (in mM) 145 KGluconate; 2 MgCl_2; 5 HEPES; 1.1 EGTA; 0.1 CaCl_2; and 5 K_2 ATP (pH 7.2; osmolality 310 mOsmol/kg H_2O). This filling solution also contained 0.5–1% biocytin for labeling neurons.

Recordings were made using an Axoclamp 2A amplifier (Axon Instruments, 3-kHz bandwidth) in bridge balance mode. During the search for a neuron, current pulses of 50–1250 pA (10–30 ms pulse width) were applied at a rate of 2 Hz. To determine the input resistance and the current-voltage relationship of a neuron, rectangular currents pulses of +60 to −110 pA (0.5–1 s pulse width) were applied and the resulting voltage deflections measured. The effects of stimulation of the dorsal roots (single-pulse stimulation, 0.1–30 V, 0.5–1 ms pulse width) were determined at a range of membrane potentials (−40 to −90 mV). Once stable responses had been obtained and

FIG. 1. A: line drawing of the preparation used in these experiments. The brain stem was pinned with the dorsal surface uppermost and the spinal cord is twisted at the cervical level so that a ventral root could be easily accessed for placement of a suction electrode. Suction electrodes were placed on a lower cervical or upper thoracic dorsal root (dr) for stimulation and an upper thoracic ventral root (vr) for recording. The patch-clamp electrode was placed on the dorsal surface of the brain stem prior to advancing into the tissue using landmarks on the surface of the brain stem as guides. B: photomicrograph of a transverse section of brain stem with a drawing of a neuron superimposed in the position of the recorded neuron within the cuneate nucleus. This technique allows the full arborizations of the filled neuron to be seen at the same focal level. The axon (arrowed) can be traced coursing ventrally within the slice with several swellings which may be due to boutons en passant. The final site of termination, however, could not be located. C: high-power photomicrograph of the same neuron. The dendrites can be clearly observed coursing mediolaterally within the cuneate nucleus with a beaded appearance. Rob, raphe obscursus; Gr, gracile nucleus; AP, area postrema; X, dorsal vagal motor nucleus; XII, hypoglossal nucleus.
various manipulations carried out, drugs were applied to determine their effects on the evoked postsynaptic potentials. All drugs were dissolved in aCSF at known concentrations and applied to the superfusing medium at a rate of 5 ml/min and as such were subject to a dead space of around 30 ml due to the volume of the bath and superfusion tube. This meant that both the effect of the drug and the washout after switching to control medium took time to occur. The following drugs were applied: the non-NMDA receptor antagonists, 6,7-dinitroquinoxaline-2,3-dione (DNQX; Research Biochemicals International) and 6-nitro-7-sulamoylbenzof[1,2-d:4,3-d']imidazoquinoline-2,3-dione (NBQX, Tocris Cookson); the NMDA receptor antagonist AP-5; the GABA_A-receptor agonist ± baclofen (both Research Biochemicals International); and the GABA_B-receptor antagonist CGP 35348 (a gift from Novartis). All drug concentrations given in RESULTS are the final concentrations in the perfusing medium.

Data analysis

Membrane potentials, current injections, whole-nerve recordings, and trigger pulses were stored via an interface (Instrutech; 11 kHz/channel sampling rate) on videotape for analysis. All data analysis was carried out using an IBM-compatible microcomputer (interface and software supplied by Cambridge Electronic Design UK). Spike durations and amplitudes were measured as the time and amplitude from the start of the rising phase of the action potential to the start of the afterhyperpolarization, respectively. Input resistance was measured at −60 mV and taken as the voltage value at the beginning of a response to a 50-pA current step. Conduction distance was measured and the axonal conduction velocity calculated for theafferent input onto each neuron. The fluctuations in onset latency of the EPSPs for each DCN neuron were determined by measuring the onset latencies of 20 single-sweep EPSPs. The mean amount by which these values varied from the mean onset latency was then calculated for each neuron (average absolute deviation). The amplitudes of the evoked postsynaptic potentials were measured as the greatest voltage deflection from the membrane potential and were calculated for each membrane potential. The voltage relationships of the fast EPSPs evoked by dorsal root stimulation were evaluated by plotting the percentage change in EPSP peak amplitude against membrane potential, taking the EPSP amplitude at −60 mV as 100%. Comparisons of the amplitudes and latencies of action potentials, input resistances, depths of neurons, afterhyperpolarizations, and voltage deflections due to activation of an I_f current were made using the Mann-Whitney U test, as were comparisons of input resistances before and after baclofen applications. All values are given as mean ± SE unless stated.

Histology

During the recording period, biocytin, which is contained within the intracellular medium, diffused into the neurons to fill them. At the end of the experiment, brain stems were fixed in 10% formal saline for up to 1 week. Eighty-micron-thick sections of brain stem were cut and filled neurons, where recovered, were photographed or reconstructed using a drawing tube.

RESULTS

Twenty-one neurons responded to dorsal root stimulation with an EPSP that occurred with a very low variation in latency (see The monosynaptic nature of the EPSP) and it is these neurons that are studied further in this report. Neurons recorded outwith the DCN either did not respond to dorsal root stimulation or responded with a polysynaptic EPSP.

Location of neurons

DCN neurons were found lateral to the area postrema at depths of 100–268 μm below the dorsal surface of the brain stem. Neurons filled with biocytin (n = 5) and recovered were located in dorsal aspect of the cuneate nucleus (according to the atlas of Paxinos and Watson 1986, see Fig. 1, B and C). Since only five filled neurons were recovered, it was not possible to correlate the anatomy of these neurons with their electrophysiological properties and their responses to dorsal root stimulation.

Electrophysiological properties of the neurons

Neurons that responded to dorsal root stimulation with a monosynaptic EPSP (n = 21) had a mean resting membrane potential of −42.2 ± 1.1 mV. The spontaneous action potentials of these neurons had a mean amplitude of 61.2 ± 1.7 mV at their resting membrane potential and a mean duration of 8.1 ± 0.4 ms. The input resistance of the neurons (measured at −60 mV) was 597 ± 70 MΩ. Current-voltage relationships were determined in 17 of the DCN neurons and in 12 of these neurons, a sag in the voltage responses to current steps was revealed at membrane potentials of more than −80 mV (see Fig. 2). This sag (measured as the difference between the instantaneous and steady-state voltage values) had a mean amplitude of 9.2 ± 1.3 mV (measured at −100 mV). The responses of these neurons to dorsal root stimulation were not different from those neurons not expressing the sag.

Positive current steps were applied to 14 neurons to reach firing threshold and differences in the shape of the action potentials could be distinguished. Six of the 14 neurons tested had action potentials that were followed by afterhyperpolarizations of mean amplitude of 13 ± 2.1 mV at firing threshold of 13 ± 2.2 mV and duration 268 ± 26 ms [see Fig. 2A(ii)]. The remaining eight neurons displayed action potentials with the repolarizing phase characterized by a transient afterdepolarization which often also reached the threshold for firing [see Fig. 2, B(ii) and C]. Where the neurons fired again in short succession, the second action potential showed a less prominent afterdepolarization (shown in Fig. 2C). This transient depolarization may involve activation of a low-threshold calcium current, as described by Jahnson and Llinas (1984) in the thalamus and recently by Canedo et al. (1998) in the DCN of the cat. The basic characteristics of these two groups of neurons did not differ significantly with regards to the amplitude and duration of the action potentials and the presence or absence of a depolarizing sag. There were no significant differences in the input resistance of the two groups of neurons and the depths at which they were recorded. Once more, there were no significant differences in the responses of the two groups of neuron to dorsal root stimulation with respect to EPSP onset latency and amplitude at −60 mV [see Fig. 2, A(i) and B(i)]. Therefore, all neurons were considered together for the remainder of this paper.

Responses of DCN neurons to dorsal root stimulation

Dorsal roots were stimulated with a single pulse and the fast EPSPs elicited were examined (see Fig. 3A). EPSPs were

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similar in amplitude and onset latency regardless of the level at which the dorsal roots were stimulated (from C6 to T3). When trains of up to eight stimuli (at frequencies of up to 100 Hz) were applied, EPSPs were observed with no failures of transmission (see Fig. 3B). At stimulus strengths just above threshold, large EPSPs could be observed often reaching the threshold for firing action potentials (see Fig. 3C). The EPSPs showed large deflections on both the rising and falling phases, suggesting activation of afferents with different conduction velocities (see Fig. 2). Indeed, as the stimulus intensity was increased further above threshold, the EPSPs increased in amplitude and the shape changed with respect to the duration and decay phase as possibly more fibers were recruited with different conduction velocities and/or some polysynaptic pathways were activated. Due to the long duration of the EPSPs, it was difficult to tell the effects of high-frequency stimulation on the later phases of the EPSP since summation of these phases occurred. The CNS is largely unmyelinated in rats of this age (Davison and Dobbing 1966), although some immature myelinated afferents are present at birth (Fitzgerald 1985) and these increase in number from days 0 to 6, the ages of the preparations used here. Thus, the intensity-related increases in EPSP amplitude are probably due to recruitment of myelinated and unmyelinated axons with different axonal diameters.

The monosynaptic nature of the EPSP

The average latency to onset of the EPSP was $14.7 \pm 1.1$ ms for all neurons tested ($n = 21$) and the conduction velocity calculated was $0.74 \pm 0.07$ m/s. For each neuron, a small variability in onset latency was observed stimulating at 2 times threshold for each neuron. The difference in onset latency from the mean onset latency was calculated for 20 single sweeps and an average was calculated for the set of neurons. This absolute average deviation was $0.17 \pm 0.02$ ms for the first phase of each EPSP, which is extremely low considering the length of the pathway being stimulated (up to 1.5 cm). This constant latency to onset of the EPSP and its ability to follow high- (up to 100 Hz) frequency stimulation suggested that at least the initial part of the EPSP was mediated by activation of a monosynaptic pathway.

The effect of membrane hyperpolarization on EPSP amplitude was determined. With hyperpolarization, the peak amplitude of the EPSPs increased in a linear fashion in all 21 of the neurons (Fig. 4A). In 11 of the neurons in this study, the descending phase of the EPSP showed a distinct deflection at more depolarized potentials, which decreased as the membrane was hyperpolarized (see Fig. 5A).

The chemical nature of the EPSP

The effects of applications of the excitatory amino acid antagonists DNQX or NBQX, which are selective for the non-NMDA receptor, were determined on six occasions. For all these DCN neurons, the dorsal root evoked EPSPs were reversibly reduced in amplitude from $14.6 \pm 2.8$ to $2.3 \pm 1$ millivolts.
mV (14.2 ± 5.7% of the control response; n = 6) by DNQX (10 μM; see Fig. 4B) or NBQX (5 μM). Recovery from this antagonism took up to 30 min to occur after return to control medium. Neither DNQX nor NBQX had any significant effect on the membrane potential or input resistance of the neurons. On all occasions, DNQX applications left a residual EPSP as observed in Fig. 4B. Therefore, on two occasions, the NMDA receptor antagonist AP-5 was also applied to look at the effect on the control response. These two neurons both exhibited an inflection on the falling phase of the EPSP (see Fig. 5A at −60 mV) that decreased with membrane hyperpolarization as described above. On both occasions, the later part of the EPSP was reduced in amplitude by applications of AP-5 and the residual EPSP was abolished by applications of DNQX or NBQX (see Fig. 5B).

**Effects of activating GABA_B receptors on the evoked response to dorsal root stimulation**

The GABA_B receptor agonist baclofen was superfused at a concentration in the bath of 1 μM onto four DCN neurons. Baclofen decreased the amplitude or abolished the EPSP evoked by dorsal root stimulation on all occasions tested. The EPSP amplitude was decreased from 16.0 ± 4.7 to 1.6 ± 0.6 mV [11.7 ± 3.3% of the control response (see Fig. 6)]. Baclofen had no significant effect on the membrane potential or input resistance of the neurons. The GABA_B receptor antagonist CGP 35348 blocked the effects of baclofen and the evoked EPSP returned to control levels within 10 min of changing to aCSF with CGP35348 (see Fig. 6).

**DISCUSSION**

This study has determined the responses of DCN neurons to stimulation of a dorsal root in a neonatal rat brain stem–spinal cord preparation. Characterization of the basic electrophysiological properties of DCN neurons revealed that these cells could be subdivided according to the shape of their action potential or the presence of a depolarizing sag in the voltage response to hyperpolarizing current pulses. However, the neurons were not grouped according to these characteristics in this study since the responses of the neurons to stimulation of a dorsal root were not significantly different. All DCN neurons

![FIG. 3. Responses of a dorsal column nucleus (DCN) neuron to dorsal root stimulation. A: three successive sweeps are superimposed to show the low fluctuation in onset latency. B: the dorsal root was stimulated at a rate of 100 Hz with twin pulses and the responses of the neuron to these stimuli are shown. The start of the first EPSP is visible and the second stimulus is superimposed on the rising phase. The EPSP elicited by this second stimulus is clearly observed showing that the neuron could respond at frequencies up to 100 Hz. C: responses of the neuron to stimulation of the dorsal root at stimulus intensities of 0.1, 0.2, 0.5, 1, 2, and 5 V. At the lower stimulus intensities, a small but stable EPSP is observed. As the stimulus intensity was gradually increased, a large EPSP was then observed.](http://jn.physiology.org/)

![FIG. 4. Voltage relationship and pharmacology of fast EPSP. A: the effect of different membrane potentials on EPSP peak amplitude. The amplitudes of EPSPs are expressed as percentages with the amplitude at −60 mV being 100%. As the neurons are hyperpolarized, the EPSP amplitudes increase. B: effect of DNQX on the dorsal root evoked response in a DCN neuron. (i): two consecutive superimposed sweeps showing the responses of the neuron to dorsal root stimulation in control medium. (ii): two consecutive superimposed sweeps of the response elicited by dorsal root stimulation in the presence of 10 μM DNQX. A small residual EPSP is observed.](http://jn.physiology.org/)
in this study responded to stimulation of a dorsal root with a fast EPSP that was mediated at least in part by activation of a monosynaptic pathway. The average conduction velocity of this pathway was 0.74 m/s, which is relatively slow in comparison to adult myelinated axons. Fitzgerald (1985) reported the presence of some immature myelinated fibers at birth and the conduction velocity of afferent fibers increased from days 0–6 as the degree of myelination increased. Therefore, it is likely that the fast EPSPs observed are due to activation of both immature myelinated and unmyelinated axons. Study of the pharmacology of these inputs revealed sensitivity to both non-NMDA and NMDA receptor antagonists. The fast EPSP could also be reduced by applications of the GABAB agonist baclofen, an effect that could be antagonized by the GABAB receptor antagonist CGP 35348. Neither of these drugs had an effect on the membrane potential or input resistance of the neurons, suggesting that the effect was due to an action at presynaptic GABAB receptors since activation of a postsynaptic site would be expected to cause membrane hyperpolarization and a change in input resistance associated with an opening of potassium channels (see Newberry and Nicoll 1984).

Detailed analysis of the onset latency of the EPSP revealed a very low fluctuation in latency from sweep to sweep (average absolute deviation was 0.17 ms). This indicates that stimulation of a dorsal root activates afferent fibers that impinge directly onto DCN neurons. In addition, DCN neurons were capable of responding to dorsal root stimulation at frequencies of up to 100 Hz, which is also indicative of activation of a monosynaptic pathway (see Inokuchi et al. 1992). Neurons responding to dorsal root stimulation in this way could be subdivided according to their action potential shape or the presence or absence of an Ih; however, the response profiles of the dorsal root evoked EPSPs were not significantly different. This indicates that all neurons, regardless of their basic electrophysiological characteristics, receive a monosynaptic input from primary afferent fibers. This confirms the observations from other studies in rat and cat (see wiring diagram in Andersen et al. 1964).

The strength of the synaptic security between individual afferent fibers and DCN neurons appears to be very high (Ferrington et al. 1986, 1987). This is further supported in this study by the observation that at stimulus strengths around threshold level the EPSP produced at threshold was often large and did not fail from sweep to sweep. As the stimulus intensity was increased, other fibers may have been recruited which caused deflections on the rising and falling phases of the EPSP at different latencies. The possibility of activating polysynaptic pathways also cannot be ruled out.

The chemical nature of the EPSP

The dorsal root evoked EPSPs could be reduced in amplitude by applications of both non-NMDA and NMDA receptor

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**Fig. 5.** Voltage relationship and pharmacology of dorsal root-evoked EPSPs in DCN neurons. A: EPSPs elicited by dorsal root stimulation at membrane potentials of −60, −70, and −80 mV. The early part of the EPSP (peak amplitude measurement, marked with an asterisk) increased in amplitude as the membrane was hyperpolarized. The later part of this EPSP shows a prominent deflection in the decay phase at the more depolarized potential (marked by the filled diamond), which is not as prominent as the neuron is hyperpolarized. B: effects of NMDA and non-NMDA receptor antagonists on the dorsal root-elicited EPSPs. Top: EPSP in control medium. Second trace: the NMDA receptor antagonist AP-5 (50 μM) added to the bathing medium caused a slight decrease in the EPSP peak amplitude and a more prominent decrease in the decay phase of the EPSP. Lower two traces: the residual EPSP elicited in the presence of AP-5 (15 min) was reduced by application of the non-NMDA receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX, 10 μM). All traces are averages of 8 responses.

**Fig. 6.** Effect of baclofen on dorsal root evoked responses. Top: response to dorsal root stimulation in control medium. Middle: responses to dorsal root stimulation in the presence of 1 μM baclofen showing that baclofen abolishes the evoked EPSP. Bottom: response in the presence of the GABAB receptor antagonist CGP53548 (100 μM) that reverses the inhibitory effect of baclofen in the medium. All traces are averages of 10–15 sweeps.
antagonists. All neurons tested responded with fast EPSPs, where the initial phase of the EPSP increased in amplitude as the membrane was hyperpolarized, consistent with the idea that the NMDA receptor plays a minimal role in this part of the EPSP. In addition, on the six occasions tested, the EPSP was reduced in amplitude by applications of the non-NMDA receptor antagonists NBQX and DNQX. This indicated that these receptors were always involved in mediating responses from primary afferent inputs. This observation is supported by the fact that both projecting and interneurons in the dorsal column nuclei of the rat express α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) receptors, although there is heterogeneity in the subtypes expressed in the different neurons (Propratiloff et al. 1997). In 11 of the 21 neurons, a distinct inflection on the falling phase of the EPSP was observed, which was reduced in amplitude as the membrane was hyperpolarized. This reduction in EPSP amplitude at hyperpolarized potentials may be due to voltage-dependent blockade by Mg$^{2+}$ of the NMDA receptor-activated channel (see Nowak et al. 1984). The later part of the EPSP was also reduced on the two occasions tested by applications of the NMDA receptor antagonist AP-5. Although the NMDA receptor antagonist was only applied twice, this observation, when taken together with the voltage relationship of the EPSP and the fact that the non-NMDA receptor antagonists did not block the EPSP fully, indicate a role for both non-NMDA and NMDA receptors in mediating the dorsal root evoked responses in some DCN neurons. However, this study does not definitively prove that NMDA receptors are located on all DCN neurons postsynaptic to primary afferent terminals.

**Inhibition of dorsal root evoked responses by baclofen**

Fast EPSPs could be reduced in amplitude by applications of the GABA$_A$ receptor agonist baclofen that had no effect on the postsynaptic membrane potential or input resistance of the neuron suggesting that the site of action was presynaptic. This is of interest since until now, observations have been concentrated mainly on the effects of activation of presynaptic GABA$_A$ receptors. It is known that primary afferents innervating DCN neurons are inhibited by GABA acting presynaptically at bicuculline sensitive sites to cause primary afferent depolarization (Davidson and Southwick 1971; Simmonds 1978). However, the role of the GABA$_B$ receptor, which can also be located presynaptically, has not been studied. Newberry and Simmonds (1984) reported a bicuculline-insensitive component of the slow negative wave in their field potential recordings from gracile nucleus when stimulating the dorsal columns that may have been due to activation of GABA$_B$ receptors. In addition, activation of GABA$_B$ receptors decreases the size of the receptive field of neurons in the DCN (Schwark et al. 1999). It is likely that GABA may have a two-fold effect on presynaptic release of excitatory neurotransmitter: 1) via the ligand-gated GABA$_A$ receptors to depolarize the terminal (Andersen et al. 1970), and 2) via GABA$_B$ receptors to prolong the presynaptic inhibitory effect since this is mediated by activation of a second messenger system (Malcangio and Bowery 1996). This double action of GABA has been observed on primary afferent input to the dorsal horn where the two GABA receptors coexist on the membrane of slowly conducting primary afferents (Desarmenien et al. 1984). The GABA$_A$ receptor activation produces primary afferent depolarizations (Curtis et al. 1971), and the GABA$_B$ receptor decreases calcium conductance presynaptically to decrease neurotransmitter release (see Malcangio and Bowery 1996).

Baclofen reduced the responses of all the DCN neurons tested to dorsal root stimulation without affecting the intrinsic properties of the postsynaptic membrane, suggesting that GABA$_B$ receptors are found on presynaptic primary afferent terminals impinging onto all neurons (see Types of DCN neuron).

**Use of the neonatal preparation: Relevance to the adult**

These data have been obtained from a neonatal rat brain stem–spinal cord preparation that enables in vitro study of the long afferent pathway onto DCN neurons. However, these preparations are taken from rats of 2–5 days in age; is the pharmacology of these inputs likely to be similar to the adult? Excitatory amino acids are known to be important in the processing of primary afferent inputs onto DCN neurons in adults (see INTRODUCTION). Furthermore, studies of the AMPA receptor distribution in adult rats show expression of AMPA subtypes in both projection neurons and interneurons, indicating that a large part of transmission in the DCN in adult involves activation of non-NMDA receptors (Propratiloff et al. 1997). This fits in well with the data presented here. The role of NMDA receptors in the DCN in adult is less well documented. In the adult mouse brain stem, the DCN expressed high levels of two NMDA receptor channel subunit mRNAs, indicative of a role for NMDA receptors in these nuclei (Watanabe et al. 1994), but to date the role of these NMDA receptors in the adult DCN is unknown. In other brain areas, e.g., the rat neocortex, NMDA receptor-mediated responses in younger animals were longer and comprised a larger component of the evoked responses than those observed in older animals (Burgard and Hablitz 1993). Lo Turco et al. (1991) observed that in the neocortex, NMDA receptors were blocked in a voltage-dependent manner by magnesium in rats at birth, although a lesser degree of voltage dependency was reported in the hippocampus (Ben-Ari et al. 1988). Our observations indicate that in the DCN of neonatal rats, NMDA receptors do play a role in the transmission of afferent inputs onto neurons and that a degree of voltage dependency is present at this age. However, it may be that with maturation, the NMDA component of the evoked response to afferent nerve stimulation decreases in the DCN.

GABA is known to be an important neurotransmitter in the DCN in the adult rat (see INTRODUCTION), although the role of GABA$_B$ receptors is less well documented. Early studies by Newberry and Simmonds (1984) showed that a component of the slow negative wave of the evoked field potential recorded in the gracile nucleus was resistant to bicuculline. In addition, GABA$_B$ receptors have recently been shown to influence the size of the receptive field of neurons in the dorsal column nuclei of rats, suggesting that these receptors play an important role in the adult DCN (Schwark et al. 1999). In the hippocampus, it has been shown that the presynaptic GABA$_B$-mediated inhibition is well developed at birth (Gaiarsa et al. 1994), although the postsynaptic GABA$_B$-mediated inhibition is poorly developed. Therefore, it is likely that the role of GABA$_B$ receptors in the presynaptic inhibition of neurotransmitter release is similar in the neonate and the adult.
Types of DCN neuron

DCN neurons showed differing characteristics according to the shape of their action potential and the presence or absence of a depolarizing sag in response to application of hyperpolarizing current pulses (indicative of activation of I_h). The different electrophysiological characteristics may be due to recording from the two main types of neuron found in the DCN, the projection neurons and interneurons (Anderson et al. 1964; Propratilloff et al. 1997), and may therefore prove to be a useful tool for identification of neurons in in vitro preparations. Indeed, in a recent in vivo study, projection neurons in the DCN displayed similar depolarizing sags while presumed interneurons did not (Canedo et al. 1998), although evidence suggested the presence of a low-threshold calcium conductance in both groups of neurons. It was not possible to distinguish between the response profiles of the different types of neuron to dorsal root stimulation, either from the shape of the EPSPs or the chemistry of the responses. However, it may be that neuromodulators other than GABA acting at GABA_B receptors will affect the DCN neurons differently.

In conclusion, these data have shown that in the neonatal rat brain stem–spinal cord preparation, fast EPSPs elicited in DCN neurons by stimulation of dorsal roots 1) have a monosynaptic component, 2) are mediated by excitatory amino acids acting on both non-NMDA and NMDA receptors, and 3) can be modulated by activation of GABA_B receptors located presynaptically. The neonatal rat brain stem–spinal cord preparation seems very suitable for the study of the pharmacology of afferent transmission onto DCN neurons.

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