Anesthesiology
76:958–966, 1992

Effects of Pentobarbital on Heterosegmentally Activated Dorsal Root Depolarization in the Rat

Investigation by Sucrose-gap Technique In Vivo

Koki Shimoji, M.D., Naoshi Fujiwara, Ph.D.,† Sadahei Denda, M.D.,‡ Misao Tomita, M.D.,‡ Makoto Toyama, M.D.,§ Satoru Fukuda, M.D.¶*

Slow positive cord dorsum (P-) potentials activated by segmental stimulation are believed to reflect primary afferent depolarizations and have been shown to be augmented by barbiturates. However, there have been no data to confirm whether heterosegmentally activated P-potentials also represent primary afferent depolarizations and are similarly affected by barbiturates. We therefore tested whether heterosegmental P-potentials reflect primary afferent depolarizations and how these heterosegmental potentials are affected by barbiturates. Heterosegmentally activated dorsal root (DR) depolarizations (depolarizations evoked in DRs of lumbar segments in response to afferent volleys to cervical segments produced by electrical stimulation of the forepaw) and P-potentials were simultaneously recorded, adapting the sucrose-gap technique for recording DR depolarization *in vivo* in the rat. Forepaw (heterosegmental) stimulations produced a large depolarization in the DRs of L5–S1 as well as a slow P-potential in the lumbosacral enlargement. Transection of the spinal cord at the level of C1–C2 abolished both the P-potential and DR depolarization activated by heterosegmental stimulation as well as the second component of segmentally (hindpaw) activated P-potential. Bicuculline (100 μg/kg, intravenous) augmented the P-potential and DR depolarization produced by heterosegmental stimulation, but larger doses, 400–600 μg/kg, eventually suppressed these. However, the drug, in a dose-dependent manner, suppressed both the P-potential and DR depolarization produced by the segmental stimulation. Pentobarbital (10–40 mg/kg, intravenous) suppressed in a dose-dependent manner both the heterosegmental P-potential and heterosegmental DR depolarization and prolonged their peak latencies. By contrast, pentobarbital augmented and prolonged the segmental P-potential and segmental DR depolarization. The second component of segmentally activated P-potential was most vulnerable to pentobarbital. Thus, the results suggest that both the P-potential and DR depolarization produced by heterosegmental stimulation mostly reflect primary afferent depolarizations produced by long feedback loops *via* supraspinal structures, and that pentobarbital suppresses heterosegmentally activated feedback inhibition and potentiates the segmental inhibition in the rat spinal cord. (Key words: Barbiturates: pentobarbital. GABA antagonist: bicuculline. Depolarization, dorsal root: heterosegmental; segmental. Potential: positive cord dorsum. Technique: sucrose-gap.)

THERE IS SOME EVIDENCE that the segmentally evoked dorsal root potential[1] or slow positive cord dorsum (P-) potential[2] reflects primary afferent depolarization[1] demonstrated by intrafiber recording.[3,4] However, stimulation of various parts of the brain has been shown to produce dorsal root potentials and inhibit interneuron activities in the dorsal horn of the spinal cord.[5–10] Besides these supraspinal controls, heterosegmental controls over the spinal dorsal horn have also been suggested.[11–13] These supraspinal and heterosegmental inhibitions are suggested to be presynaptic in nature,[5,8,12] because dorsal root potentials, believed to reflect primary afferent depolarization, are produced.

This laboratory has demonstrated previously that P-potentials, produced in the lumbosacral enlargement, can also be recorded after descending volleys in humans[14,15] and by heterosegmental stimulations in rats,[16,17] suggesting that these slow positive waves reflect primary afferent depolarization. γ-Aminobutyric acid (GABA) is believed to be the neurotransmitter responsible for segmentally activated primary afferent depolarization in amphibian and cat spinal cords.[18] However, there have been no data regarding the effects of barbiturates on heterosegmentally activated primary afferent depolarization in the intact spinal cord.

The present study had two objectives, for which we used simultaneous recordings of lumbar dorsal root (DR) depolarization with an application of the *in vivo* sucrose-gap technique in the spinal cord of rat. First, we wished to reconfirm whether the P-potentials of lumbar spinal cord activated by heterosegmental (forepaw) stimulations actually reflect primary afferent depolarizations. Second, we studied how both the heterosegmental DR depolarization and heterosegmental P-potential (forepaw stimulation) are affected by pentobarbital in comparison to segmental DR depolarization and segmental P-potential (hindpaw stimulation).

Materials and Methods

The approval of the institutional committee on animal experimentation was obtained, and the institutional

* Professor of Anesthesiology.
† Instructor of Anesthesiology.
‡ Assistant of Anesthesiology.
§ Research assistant of Anesthesiology.
¶ Associate Professor of Anesthesiology.

Received from the Laboratory of Clinical Neurophysiology, Department of Anesthesiology, Niigata University School of Medicine, Niigata, Japan. Accepted for publication January 23, 1992. Supported in part by a grant-in-aid (02557058) from the Japanese Ministry of Education, Science and Culture.

Address reprint requests to Dr. Shimoji: Laboratory of Clinical Neurophysiology, Department of Anesthesiology, Niigata University School of Medicine, #1-757 Asahi-machi, Niigata 951, Japan.

guidelines for use of laboratory animals were observed and followed during all aspects of this study.

The experiments were carried out on 16 adult male rats weighing 350–400 g. Anesthetic and surgical procedures were the same as in a previous study[16] except for the introduction of the *in vivo* sucrose-gap technique. Following an intraperitoneal injection of 120 mg/kg ketamine hydrochloride with 0.1 mg/kg atropine sulfate, a tracheal tube was introduced through a tracheotomy and connected to a Harvard animal ventilator. Ventilation volume was adjusted to maintain a normal $P_{ET_{CO_2}}$ at 35–40 mmHg. The animals were paralyzed by intravenous injections of pancuronium bromide (0.2 $mg \cdot kg^{-1} \cdot h^{-1}$).

The left femoral artery and vein were cannulated to monitor arterial blood pressure (see tables 2 and 3) or sample blood gases and to permit the infusion of Ringer's lactate solution (4 $ml \cdot kg^{-1} \cdot h^{-1}$) with ketamine (30 $mg \cdot kg^{-1} \cdot h^{-1}$) and other drugs (bicuculline and pentobarbital). Preliminary experiments with electroencephalography and somatosensory evoked potentials from the brain and spinal cord showed no significant changes with stable arterial blood pressure over a period of several hours at this infusion rate of ketamine.[16] This infusion rate of ketamine was adequate for suppressing the behavioral responses of the rat to surgical incision of the skin without administration of a muscle relaxant. A cannula was also inserted through a small skin incision into the urinary bladder for monitoring urine volume. The electrocardiogram was monitored continuously through needle electrodes inserted into the subcutaneous tissue of the thorax. Rectal temperature was maintained at 37.0–38.0° C by a homeothermic blanket system.

The animals were mounted on a stereotactic frame, and laminectomies were carried out on T12–L2 vertebrae to expose the lumbar cord. The head was also gently secured in the frame by ear bars. The spinal cord was exposed and superfused with prewarmed (37° C) Krebs solution of the following composition (millimolar): NaCl 117, KCl 3.6, NaH_2PO_4 1.2, $CaCl_2$ 2.5, $MgCl_2$ 1.2, glucose 11, and $NaHCO_3$ 25.

To confirm whether the P-potentials activated by heterosegmental stimulations reflect DR depolarization, we used a sucrose-gap technique *in vivo*, for purely detecting lumbar DR depolarizations, and recorded the DR depolarizations simultaneously with P-potentials. Because depolarization of the primary afferent terminals is believed to spread electrotonically with sharp decay in amplitude as a function of distance from the terminals,[2] it was desirable to place the sucrose-gap wall as close as possible to the entry of the roots on the surface of the spinal cord to keep the amplitude as large as possible. For this purpose, a special apparatus was constructed (fig. 1).

The apparatus was composed of three chambers containing sucrose, Krebs solution, and paraffin oil (fig. 1).

TABLE 1. Waveform Characteristics of Dorsal Root Depolarization and Cord Dorsum Slow Positive Potential Produced in Lumbar Spinal Cords of 16 rats *in vivo*, by Segmental (Hindpaw) and Heterosegmental (Forepaw) Stimulations

	Electrical Activity	Mean ± SE	Significance
Segmental stimulation			
Onset latency* (ms)	DRdep	8.9 ± 0.7	NS
	P-pt	8.4 ± 0.4	
Peak latency (ms)	DRdep	33.3 ± 4.2	NS
	P-pt	25.9 ± 2.5	
Amplitude	DRdep(mV)	1.6 ± 0.2	
	P-pt (μV)	45.1 ± 6.6	
Duration† (ms)	DRdep	128.6 ± 10.4	$P < 0.01$
	P-pt	95.4 ± 7.2	
Heterosegmental stimulation			
Onset latency* (ms)	DRdep	20.4 ± 0.9‡	$P < 0.01$
	P-pt	16.4 ± 0.4‡	
Peak latency (ms)	DRdep	52.5 ± 3.5‡	$P < 0.01$
	P-pt	34.1 ± 2.5‡	
Amplitude	DRdep(mV)	1.2 ± 0.3	
	P-pt (μV)	35.9 ± 7.4	
Duration† (ms)	DRdep	121.6 ± 11.8	$P < 0.01$
	P-pt	82.9 ± 6.8	

* Onsets of the dorsal root depolarization (DRdep) and cord dorsum slow positive potential (P-pt) in response to segmental stimulation were determined by the beginning of the positive-going phase of the preceding negative wave (see fig. 2).

† DRdep and P-pt were recorded by a high impedance DC amplifier and an AC amplifier (time constant 2.0 s), respectively (see Materials and Methods). Differences in each parameter were calculated between the DRdep and P-pt (P values in the right column), and also between those activated by segmental and heterosegmental stimulation.

NS = no significant difference.

‡ $P < 0.01$.

The sucrose chamber (3 × 10 × 4 mm) was bathed with an isotonic (339 mOsm) sucrose solution at a rate of 15 ml/min. The Krebs chamber (3 × 14 × 4 mm) was also superperfused with Krebs solution at a rate of 10 ml/min. The third chamber, of the same size as the Krebs chamber, was filled with paraffin oil. The bottom of the sucrose chamber was covered with a thin rubber skin through which a small hole (approximately 0.3 mm) was made to introduce a rootlet of the lumbosacral DR (L5–S1). The rootlet was further led to the other two chambers, also through small holes made in thin plastic walls dividing the chambers. Small spaces between the rootlet and the small holes were sealed gently with petroleum jelly.

Completeness of the sucrose-gap was confirmed by conduction block of nerve impulses by the gap, *i.e.*, disappearance of the cord dorsum potentials recorded from the DR entry zone despite preservation of a monophasic spike potential detected by the distal electrode for recording DR depolarization submerged in the Krebs solution in response to DR stimulation (see fig. 2).

TABLE 2. Effects of Intravenous Administrations of Bicuculline on Segmentally or Heterosegmentally Activated Dorsal Root Depolarization and Cord Dorsum Slow Positive Potential

		Bicuculline (μg/kg, Intravenous)				
		Control	100	200	400	600
Arterial plasma concentration (ng/ml)			<20	129 ± 42	342 ± 37	913 ± 26
Heart rate beats/min		428 ± 9	420 ± 11	408 ± 7	412 ± 13	431 ± 17
Mean arterial pressure (mmHg)		97 ± 4	115 ± 8	120 ± 7	128 ± 5	132 ± 7
Segmental (hindpaw) stimulation						
Peak latency (%)	DRdep	100	92 ± 6	95 ± 6	91 ± 8	92 ± 7
	P-pt	100	98 ± 7	94 ± 7	97 ± 5	98 ± 4
Amplitude (%)	DR dep	100	74 ± 8*	39 ± 7*	18 ± 4†	4 ± 3‡
	P-pt	100	72 ± 9*	36 ± 8*	17 ± 3†	4 ± 5‡
Duration (%)	DRdep	100	88 ± 7	89 ± 8	88 ± 9	82 ± 8
	P-pt	100	86 ± 8	94 ± 7	91 ± 7	85 ± 8
Heterosegmental (forepaw) stimulation						
Peak latency (%)	DRdep	100	112 ± 7	121 ± 9	129 ± 5*	133 ± 4†
	P-pt	100	108 ± 6	114 ± 7	131 ± 8*	138 ± 5†
Amplitude (%)	DR dep	100	222 ± 26*	114 ± 42	78 ± 12*	22 ± 8†
	P-pt	100	210 ± 23*	180 ± 45	82 ± 8*	17 ± 7†
Duration (%)§	DRdep	100	113 ± 7	124 ± 11	118 ± 12*	115 ± 8*
	P-pt	100	111 ± 8	119 ± 9	113 ± 7*	108 ± 7*

Calculations of the records were carried out 0.5–1.5 min after intravenous injection of the drug and are expressed as a percent of the control value (mean ± SE; n = 7).

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$ (n = 7 in all values) as compared to the control value. There were no significant differences in each parameter between dorsal root depolarization (DRdep) and cord dorsum slow positive potential (P-pt) at all different doses of the drug.

§ Comparison was made by the half decay time. Arterial plasma concentrations of bicuculline at 0.5–1.0 min are presented.

Flexible ball-tip electrodes were placed on the lumbar (L5) cord surfaces at the midline. Reference electrodes were fixed to nearby bones. Two needle electrodes were placed at the left frontal and occipital areas to monitor the electroencephalogram (EEG) and cortical evoked potentials. The right fore- and hindpaws were electrically stimulated by a stimulator (Nihonkohden SEN-7103) through an isolation unit at 1 Hz with silver/silver chloride needle electrodes (150 μm in diameter, 10 mm in length), inserted subcutaneously into the first and fifth

TABLE 3. Effects of Pentobarbital on Segmentally or Heterosegmentally Activated Dorsal Root Depolarization and Cord Dorsum Slow Positive Potential

		Pentobarbital (mg/kg)				
		Control	10	20	30	40
Aterial plasma concentration (μg/ml)			13.7 ± 0.4		42.0 ± 2.9	
Heart rate beats/min		420 ± 16	394 ± 15	334 ± 30	311 ± 23	292 ± 18
Mean arterial pressure (mmHg)		86 ± 6	64 ± 3	51 ± 9	48 ± 9	45 ± 4
Segmental (hindpaw) stimulation						
Peak latency (%)	DRdep	100	145 ± 6*	162 ± 5†	187 ± 7‡	212 ± 8‡
	P-pt	100	148 ± 8*	159 ± 7	185 ± 8‡	198 ± 7‡
Amplitude (%)	DRdep	100	141 ± 6*	198 ± 7†	209 ± 6‡	160 ± 4‡
	P-pt	100	143 ± 4†	196 ± 8†	218 ± 7‡	156 ± 3‡
Duration (%)§	DRdep	100	140 ± 6*	171 ± 5†	189 ± 6‡	220 ± 7‡
	P-pt	100	138 ± 5*	168 ± 6†	192 ± 7‡	218 ± 11†
Heterosegmental (forepaw) stimulation						
Peak latency (%)	DRdep	100	133 ± 4†	171 ± 6†	198 ± 7‡	—
	P-pt	100	137 ± 5†	179 ± 7†	211 ± 9†	—
Amplitude (%)	DRdep	100	82 ± 4*	32 ± 4‡	9 ± 2‡	0‡
	P-pt	100	78 ± 6*	29 ± 5†	11 ± 3‡	0‡
Duration (%)§	DRdep	100	134 ± 4*	142 ± 8†	—	—
	P-pt	100	132 ± 5*	147 ± 9†	—	—

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$ (n = 7 in all values) as compared to the control value. There were no significant differences in each parameter between dorsal root depolarization (DRdep) and cord dorsum slow positive potential (P-pt) at all different doses of the drug.

Calculations of the records were carried out 2–5 min after intravenous injection of the drug and are expressed as a percent of the control value (mean ± SE; n = 7).

§ Comparison was made by the half decay time. Arterial plasma concentrations of pentobarbital (following 10 and 30 mg/kg) at 2 min are presented.

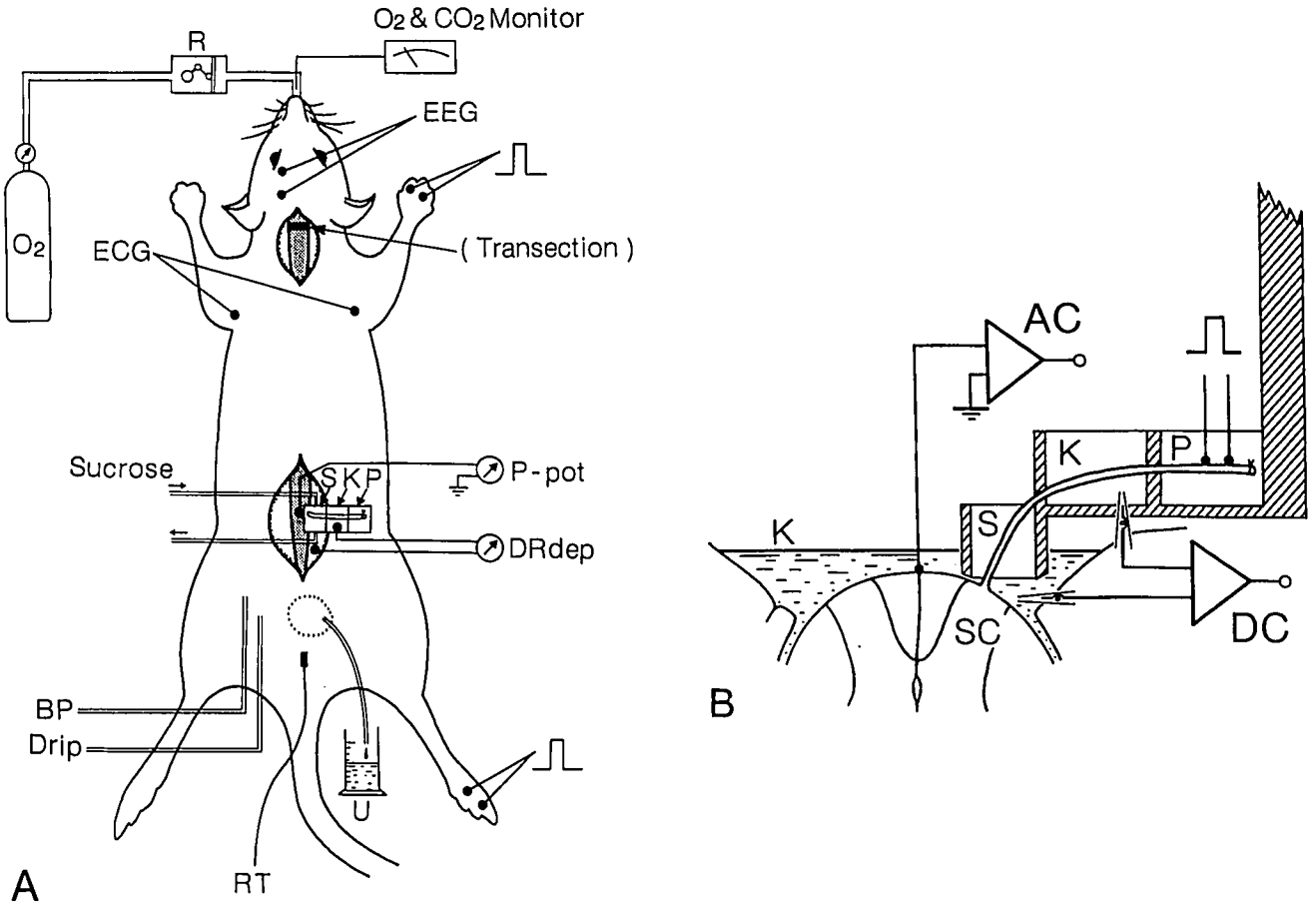

FIG. 1. *A:* General arrangements for recording lumbar dorsal root depolarization (DRdep) and positive cord dorsum potential (P-pot) produced by forepaw (heterosegmental) and hindpaw (segmental) stimulations. R = respirator; EEG = electroencephalogram; ECG = electrocardiogram; S (= sucrose), K (= Krebs), and P (= paraffin oil) = three small chambers for the sucrose-gap (see B for details); BP = direct arterial blood pressure measurement; Drip = a catheter indwelled in the left femoral vein for drip infusion of Ringer's lactate solution and drugs; RT = rectal temperature; U = urine volume measurement (see text for details). *B:* Schematic presentation of the *in vivo* sucrose-gap technique developed for the present study to record lumbar dorsal root depolarizations. The bottom of the sucrose chamber (S) was covered by a thin rubber membrane placed close to the lumbar dorsal root entry zone. The lumbar dorsal root was led through small (0.3 mm in diameter) holes made in the rubber membrane and plastic walls to the S, K, and P chambers, respectively. SC = spinal cord.

digits. Stimulus (square wave monophasic pulse, 0.1 ms in duration) intensity was adjusted to approximately 25 × threshold strength for the segmental negative cord dorsum potential so that it constantly produced heterosegmental slow positive waves in all animals. The same stimulus was applied to both fore- and hindpaws using a switch box. The stimulus intensity used was monitored in three animals by recording the nerve action potentials from the sural nerve at the end of the experiments, and verified to evoke Aβ- and Aδ-fiber action potentials but not C-fiber action potentials.[17]

For recording the DR depolarization, a high-impedance (more than 10,000 MΩ) DC amplifier (Nihonkohden MEZ-8101) was used. In the preliminary experiments, we checked the waveforms of the P-potential recorded by the DC amplifier and an AC biophysical amplifier with the time constant set at 2.0 s, and found no important differences in the time course of the potentials. All potential changes were averaged (n = 50) by a computer (ATAC 1300, Nihon Kohden) and plotted on an x–y plotter.

In our preliminary experiments, bicuculline (intravenous) produced an enormous seizure activity in the EEG and a transient hypertension followed by a prolonged hypotension at a dose of more than 800 μg/kg. Therefore, to minimize possible hemodynamic side effects, we limited the doses of bicuculline to 100, 200, 400, and 600 μg/kg and used only two of the four doses in each experiment in reference to the table of random (sampling) numbers. Bicuculline was administered through the left femoral vein with an interval of more than 30 min between doses. Pentobarbital then was injected, with an interval of more than 1 h between doses in each animal. There was complete recovery between the bicuculline doses and partial-to-

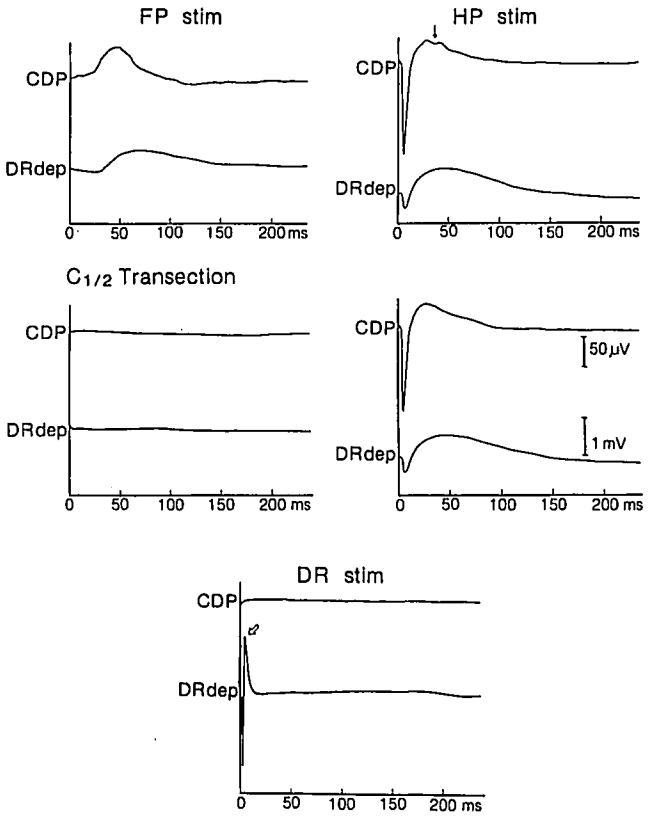

FIG. 2. Cord dorsum slow positive potential (CDP) and dorsal root depolarization (DRdep), recorded by means of the *in vivo* sucrose-gap technique, in response to segmental (hindpaw [HP]) and heterosegmental (forepaw [FP]) stimulations in a rat. Transection of the spinal cord at the C1–C2 level (middle graphs) completely abolished the heterosegmental CDP and DRdep, but did not significantly change the segmental CDP and DRdep except for the disappearance of the secondary component of the positive potential. Dorsal root stimulation (10 × threshold strength) evoked only a spike potential (*open arrow*) in the dorsal root without producing CDP or DRdep, clearly indicating that conduction block of the nerve impulse by the sucrose-gap was complete (the initial downward deflection: the stimulus artifact). Each trace represents an average of 50 responses in this and subsequent figures. Positivity in CDP and depolarization of the root are shown as upward deflection in this and subsequent figures. The start of each trace coincides with the stimulation (zero on abscissa). *Vertical arrow:* a negative dip often observed in an *in situ* preparation (*top right*), indicating the presence of the second component of the segmental positive potential that disappeared after transection of the spinal cord at the C1–C2 level (*middle right*). Note the difference in amplitude scale between CDP (50 μV) and DRdep (1 mV) in this and subsequent figures.

complete recovery (70–85% of the control value) between the pentobarbital doses. Arterial blood concentration of bicuculline and pentobarbital was measured by sampling through a catheter cannulated into the left femoral artery. 0.2 ml of the blood was sampled sequentially at 0.5–1, 2, 5, 10, 20, and 30 min after intravenous injection of the drugs. Arterial plasma concentrations were measured after all doses of bicuculline and after the 10- and 30-mg/kg doses of pentobarbital.

Sampled blood was centrifuged at 3,000 rpm for 10 in, and plasma was separated and stored in the refrigerator at −80° C until analysis. For bicuculline analysis, 0.1 ml of the defrosted plasma was mixed with 0.1 ml methanol and 0.05 ml acetonitrile, and the mixture was centrifuged at 3,000 rpm for 10 min. The supernatant (0.15 ml) was injected into a high-performance liquid chromatography system (Shimadzu LC-3A) equipped with a μBondapak CN column (Waters) and an ultraviolet-spectrophotometric detector (Shimadzu SPD-6A). A mobile phase consisting of 10 mM phosphate buffer 66 vol%, methanol 10 vol%, and acetonitrile 24 vol%, adjusted at *p*H 4.0, was eluted at 1.5 ml/min. The absorbance at 222 nm was monitored for bicuculline analysis. Analytical data were stored and calculated with a data analyzer (Shimadzu C-R3A). More than 20 ng/ml of bicuculline in plasma was detectable by this method.

For pentobarbital analysis, 0.05 ml of the defrosted plasma was mixed with 0.05 ml methanol and 0.15 ml acetonitrile, and the mixture was centrifuged at 3,000 rpm for 10 min. An aliquot (0.05 ml) of the supernatant was injected to the same high-performance liquid chromatography system with a μBondapak C_{18} column (Water). Eluate was the same solution as used in bicuculline analysis, and flow rate was 2.0 ml/min. The absorbance was monitored at 206 nm. Detection limit of pentobarbital in plasma was 1 μg/ml by this method.

A two-way analysis of variance was conducted to identify significant differences in amplitude, latency, and duration changes in the DR depolarization and P-potentials after drug administrations. After this analysis, the least significant difference test for multiple comparison[19] was carried out when significant differences were found. With 40 mg/kg pentobarbital, the amplitudes of both the DR depolarization and P-potential activated by heterosegmental stimulation diminished nearly to zero. For data analysis, these amplitudes were considered less than 1% of control, and Mann-Whitney U nonparametric tests[20] were conducted to calculate statistical significance from the control values. The durations of the DR depolarization and P-potential activated by heterosegmental stimulation at 30 mg/kg and their peak latencies and durations at 40 mg/kg pentobarbital sodium were not included in the data analysis, because the smallness of the potentials made it difficult to calculate these parameters. A *P* value less than 0.05 was considered significant.

Results

Stimulation of the DR being tested produced only a spike potential detected by the distal electrode in the sucrose-gap recording, without provoking any potential changes in the spinal cord. This indicated a complete block

of the afferent volley along the root by the sucrose-gap technique (fig. 2). Segmental (hindpaw) stimulation produced a large and slow potential change in a rootlet of the lumbosacral DRs tested (L5–S1) (segmental DR depolarization) and, at the same time, produced a P-potential (segmental) (fig. 2). Heterosegmental (forepaw) stimulation also produced a slow depolarization of the lumbosacral DRs (heterosegmental DR depolarization) as well as a P-potential (heterosegmental) on the dorsal surface of the lumbar cord (fig. 2).

Spinal transection at C1–C2 abolished both the heterosegmental DR depolarization and the heterosegmental P-potential. There were no substantial changes in the segmental DR depolarization and segmental P-potential, but disappearance of the second component of the segmental P-potential (fig. 2) in all five rats tested was noted. Waveform characteristics of segmental and heterosegmental DR depolarizations as well as P-potentials are summarized in table 1. Onset and peak latency and duration of the heterosegmental DR depolarization were longer than those of the heterosegmental P-potential. Duration of the segmental DR depolarization also was longer than that of the segmental P-potential. Onset and peak latencies of both the heterosegmental DR depolarization and the heterosegmental P-potential were longer than those of the segmental DR depolarization and segmental P-potential, respectively, as expected (table 1). The basis for expecting the latencies to be longer for the heterosegmental potentials is the increased conduction distance and synaptic delays in the supraspinal structures that are presumed to generate them.

Intravenous bicuculline suppressed both the segmental DR depolarization and segmental P-potential in a dose-dependent and reversible manner (table 2), whereas smaller doses (100 μg/kg) of the drug augmented, medium doses (200 μg/kg) variously affected, and larger doses (400–600 μg/kg intravenous) transiently suppressed both the heterosegmental DR depolarization and the heterosegmental P-potential (fig. 3, table 2). The suppression (0.5–1.5 min after the drug) by bicuculline of DR depolarization and P-potential evoked by heterosegmental stimulation, however, was always followed by facilitation (2–10 min after the drug) (data not shown).

Pentobarbital (10–40 mg/kg, intravenous) augmented both the segmental DR depolarization and segmental P-potential and prolonged their latencies and durations. Augmenting effects of pentobarbital on the amplitudes of both the segmental DR depolarization and segmental P-potential were maximal at 30 mg/kg (table 3). In contrast, the heterosegmental DR depolarization was delayed and diminished in amplitude at doses of pentobarbital that had parallel effects on the heterosegmental P-potential (fig. 4 and table 3).

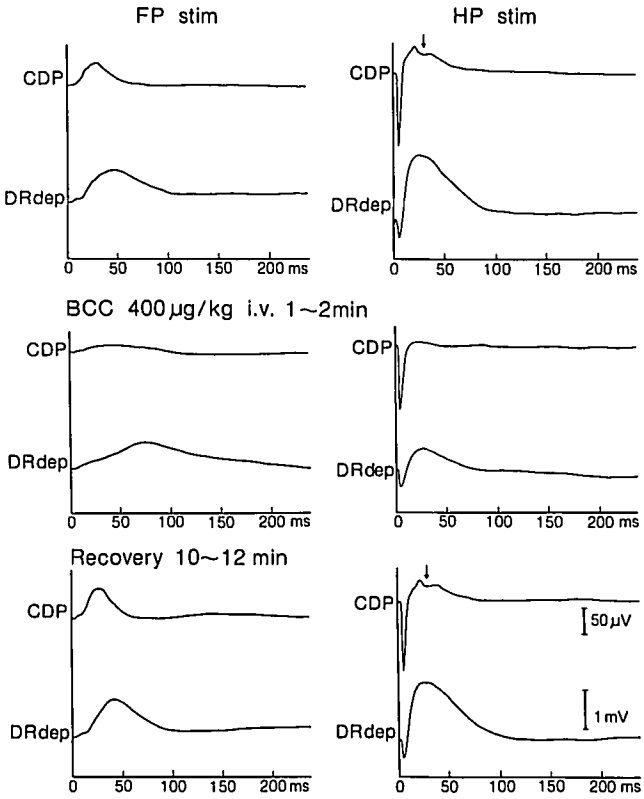

FIG. 3. Effects of intravenous administrations of bicuculline (BCC) (400 μg/kg) on cord dorsum slow positive potential (CDP) and dorsal root depolarization (DRdep) in the lumbar cord of a rat. Note the profound suppression by the drug of both CDP and DRdep activated by segmental (Hp stim) as well as heterosegmental (FP stim) volleys. Vertical arrows indicate negative dips (as in fig. 1), the second components of the segmental positive potential, which were vulnerable to both BCC and pentobarbital. These recordings were made from the same rat as presented in figure 2.

Discussion

The present results demonstrate that both P-potential and DR-depolarization can be simultaneously produced in the lumbosacral cord of rats by heterosegmental (forepaw) stimulations. Both of the potentials disappeared after high spinal transection, suggesting that feedback loops *via* supraspinal structures are responsible for producing these heterosegmentally activated slow potential changes. Alternatively, the cervical section may interfere with the afferent volley in the dorsal horn supplying the forepaw, thus reducing the effect of propriospinal mechanisms. However, the first explanation may be more valid, since the onset latencies of both the heterosegmental DR depolarization and the P-potential were considerably long (table 1) and variable. Moreover, our previous study[17] showed that the cord dorsum P-potentials recorded at the C5 level by electrical stimulation of the forepaw was not

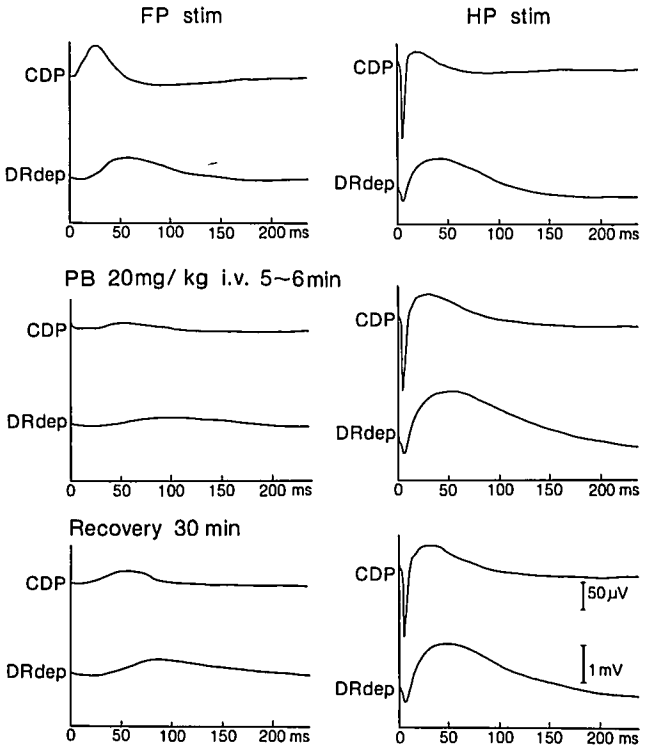

FIG. 4. Effects of pentobarbital sodium (PB) on segmentally (hindpaw [HP]) and heterosegmentally (forepaw [FP]) activated cord dorsum slow positive potential (CDP) and dorsal root depolarization (DRdep). Note the augmentation by PB of both the DRdep and CDP activated by segmental volley but the profound suppression by the drug of heterosegmentally activated DRdep and CDP. Note also that the second component of the segmental positive potential was not recorded in this rat.

affected after the cervical section. The disappearance of the second component of the segmental P-potential with the cervical section similarly suggests that this component also originates from feedback loops *via* supraspinal structures, as suggested in our previous report.[17] Furthermore, the similar responses of both heterosegmental P-potential and heterosegmental DR-depolarization to intravenous injection of bicuculline (GABA antagonist) and pentobarbital (GABA agonist) suggest that most parts of both potentials are similar in origin, *i.e.*, primary afferent depolarization activated by feedback loops via supraspinal structures.

Both the slow P-potential and the DR-depolarization produced by segmental (hindpaw) stimulation decreased in response to bicuculline and increased in response to pentobarbital. Although there has been some evidence that both P-potential and negative DR potential (DRP-V by Lloyd's terminology)[1] activated by segmental nerve or DR stimulation reflect primary afferent depolarization,[3,4,21-24] a recent study suggested that inhibitory postsynaptic potentials are also partially involved in the segmental P-potential.[25] The present study demonstrated that the segmental and heterosegmental P-potentials are not the same either in onset or peak latency or in duration as the segmental and heterosegmental DR depolarizations, respectively (table 1). Nevertheless, a similar dose-dependent blockade by bicuculline of both the segmental DR depolarization and segmental P-potential in the present study strongly suggests that a major part of the P-potential originates from primary afferent depolarization. It has been suggested that GABA is the transmitter responsible for producing primary afferent depolarization, by increasing the chloride conductance of the membrane of primary afferent terminals.[25-30] Thus, the present results also support our previous hypothesis[17] that heterosegmentally activated P-potentials of the rat spinal cord may reflect a feedback primary afferent depolarization.

Systemic administration of bicuculline caused generalized excitement or convulsion, as demonstrated in the present study by EEG (data not shown) as well as by evoked potentials. At the same time, both the segmental DR depolarization and segmental P-potential were profoundly suppressed by systemic bicuculline administration (fig. 3 and table 2). The results suggest that the drug decreases presynaptic inhibition at the spinal level, resulting in augmentation of afferent impulses and generalized excitement. Plasma concentrations of bicuculline decreased so fast that these transient inhibitions by larger doses of the heterosegmentally activated potentials were eventually followed by augmentation and then returned to the control level. For instance, arterial plasma concentrations of bicuculline at the doses of 400 and 600 μg/kg were already undetectable 20 and 30 min after the administration, respectively.

Besson and Rivot[12] recorded DR potentials and interneuron firings in response to heterosegmental and heterosensory stimuli in chloralose-anesthetized cats and suggested that presynaptic controls of supraspinal origins are mediated by their convergent interneurons. It has long been believed that both cord dorsum P-waves and DR potentials in response to segmental nerve or root stimulations reflect primary afferent depolarization, as indices of presynaptic inhibition. In fact, Koketsu[3] and Eccles and Krnjevic[4] showed direct evidence, by intrafiber recording, that the segmental DR potential (DRP-V by Lloyd's terminology)[1] represents primary afferent depolarization. However, there has been no direct evidence that heterosegmentally activated cord dorsum P-potentials or DR potentials also represent primary afferent depolarization. It is strongly suggested by the present sucrose-gap technique *in vivo* that the P-wave activated by heterosegmental stimulations largely reflect primary afferent depolarization activated by a feedback loop *via* supraspinal structures.[17]

Our hypothesis that the heterosegmental P-potential largely reflects primary afferent depolarization is based on our various findings. First, its waveform and time-course are similar to the segmental P-potential and segmental negative DRP (table 1). Second, its time-course is almost the same to that of inhibition of wide dynamic range neurons activated by heterosegmental stimulation.[17] Third, DR depolarization could be concomitantly produced by heterosegmental stimulation, as demonstrated in the present study. Fourth, it disappeared completely by transection of the spinal cord at the level of C1–C2, as shown in the present and previous studies.[16,17] Fifth, both the heterosegmental DR depolarization and heterosegmental P-potential behaved similarly in response to the drugs, as demonstrated in the present study.

The effects of anesthetics on inhibitory activities have been reported as both enhancement and as depression.[16,31–37] This inconsistency in the effects of anesthetics on inhibitory potentials might come from the differences in doses or specificity of each anesthetic or from differences in the location of synapses in the central nervous system. For instance, evoked inhibitory responses of mesencephalic reticular neurons and inhibitory postsynaptic potentials of hippocampal CA1 neurons are more vulnerable to inhalational anesthetics than evoked excitatory responses of mesencephalic reticular formation neurons[32] and excitatory postsynaptic potentials of the CA1 neurons,[33] respectively. Furthermore, there are at least three inhibitory mechanisms at the synaptic levels (postsynaptic, presynaptic, and recurrent), and there may also be feedback and other unknown inhibitory processes in integrated central nervous systems. How each anesthetic acts on these individual inhibitory activities remains to be investigated.

In contrast, barbiturates always potentiate the evoked inhibitory responses of mesencephalic reticular formation neurons,[34] inhibitory postsynaptic potentials of hippocampal CA1 neurons,[35] and P-potentials of human spinal cord, which have been suggested to be a reflection of presynaptic inhibition.[36] The underlying mechanism for those potentiating effects of barbiturates on both presynaptic and postsynaptic inhibitory potentials has variously been interpreted, as, for instance, the result of either a decrease in GABA uptake to the presynaptic terminal (see ref. 29) or an increase in the affinity of the receptor for GABA.[37] It has been suggested that GABA is the neurotransmitter of both inhibitory postsynaptic potentials and presynaptic inhibition.[22,23,27,28,31] However, there have been no data to show whether the slow depolarization produced by a feedback loop as demonstrated in the present study is caused by GABA. Therefore, we could not attribute the depressant effect of pentobarbital on the amplitudes of both the heterosegmental DR depolarization and heterosegmental P-potential to a certain central nucleus that may send the feedback impulses to the spinal cord[28] or to spinal dorsal horn interneurons that produce both the heterosegmental DR depolarization and heterosegmental P-potential.

The present study showed that pentobarbital augmented the segmentally activated DR depolarization and P-potential but inhibited the heterosegmentally produced DR depolarization and P-potential. These differential actions of pentobarbital on the heterosegmental DR depolarization (and P-potential) and segmental DR depolarization (and P-potential) may reflect corresponding actions on spinal inhibition during anesthesia with barbiturates. A nearly complete recovery of these potentials occurred in 60 min at the dose of 10 mg/kg, and partial recovery (70–85%) was achieved even in more than 1 h at the doses of 20–40 mg/kg of the drug. Half decay times of pentobarbital in arterial plasma at the doses of 10 and 30 mg/kg were approximately 5 (9.7 ± 0.8 μg/ml) and 30 min (23.1 ± 2.7 μg/ml), respectively. This may indicate prolonged effects of pentobarbital on the central nervous systems in contrast to short actions of bicuculline.

These effects of bicuculline and pentobarbital on spinal electrical activities are not believed to be caused by hemodynamic changes, since neither hypertension caused by dopamine (mean arterial pressure 160–180 mmHg) nor hypotension caused by infusion of trimetaphan (mean arterial pressure 40–50 mmHg) caused any significant changes in the spinal electrical activities tested in 3 rats (data not shown), as demonstrated also in our previous report.[16]

Although several recent studies have used the sucrose-gap technique in isolated amphibian[38–41] and even mammalian[30] spinal cords, there have been no such studies *in vivo*. The apparatus applied in the present experiment might be useful for recording the DR depolarization *in vivo* in small animals such as the rat. Thus, the present methods of recording the DR depolarization *in vivo* could be applied in similar pharmacologic studies.

The authors thank Dr. W. D. Willis, Dr. S. Nishi, and Dr. H. Higashi for their kind criticisms and suggestions for this study. The authors also thank Mr. Y. Sato for his technical expertise in assisting this experiment.

References

1. Lloyd DPC: Electrotonus in dorsal nerve roots. Cold Spring Harb Symp Quant Biol 17:203–219, 1952
2. Eccles JC: The Physiology of Synapses. New York, Springer-Verlag, 1964, pp 220–238
3. Koketsu K: Intracellular potential changes of primary afferent nerve fibers in spinal cords of cats. J Neurophysiol 19:375–392, 1956

4. Eccles JC, Krnjevic K: Potential changes recorded inside primary afferent fibers within the spinal cord. J Physiol (Lond) 149:250–273, 1959
5. Andersen P, Eccles JC, Sears TA: Cortically evoked depolarization of primary afferent fibers in the spinal cord. J Neurophysiol 27: 63–77, 1964
6. Carpenter D, Engberg I, Lundberg A: Primary afferent depolarization evoked from the brain stem and the cerebellum. Arch Ital Biol 104:50–72, 1966
7. Lundberg A, Vyklicky L: Inhibition of transmission to primary afferents by electrical stimulation of the brain stem. Arch Ital Biol 104:86–97, 1966
8. Proudfit HK, Anderson EG: New long latency bulbo-spinal evoked potentials blocked by serotonin antagonists. Brain Res 65:542–546, 1974
9. Mokha SS, Iggo A: Mechanisms mediating the brain stem control of somatosensory transmission in the dorsal horn of the cat's spinal cord: An intracellular analysis. Exp Brain Res 69:93–106, 1987
10. Martin RF, Haber LH, Willis WD: Primary afferent depolarization of identified cutaneous fibers following stimulation in medial brain stem. J Neurophysiol 42:779–790, 1979
11. Besson JM, Rivot JP: Heterosegmental, heterosensory and cortical inhibitory effects on dorsal interneurones in the cat's spinal cord. Electroenceph Clin Neurophysiol 33:195–206, 1972
12. Besson JM, Rivot JP: Spinal interneurones involved in presynaptic controls of supraspinal origion. J Physiol (Lond) 230:235–254, 1973
13. Benoist JM, Besson JM, Conseiller C, Le Bars D: Action of bicuculline on presynaptic inhibition of various origins in the cat's spinal cord. Brain Res 43:672–676, 1972
14. Shimizu H, Shimoji K, Maruyama Y, Matsuki M, Kuribayashi H, Fujioka H: Human spinal cord potentials produced in lumbosacral enlargement by descending volleys. J Neurophysiol 48: 1108–1120, 1982
15. Shimoji K, Fujioka H, Maruyama Y, Shimizu H, Hokari T, Takada T: Spinal cord potentials (SCPs) produced by descending volleys in man, Neurophysiology and Standards of Spinal Cord Monitoring. Edited by Ducker TB, Brown RH. New York, Springer-Verlag, 1986, pp 114–121
16. Shimoji K, Fujiwara N, Fukuda S, Denda S, Takada T, Maruyama Y: Effect of isoflurane on spinal inhibitory potentials. ANESTHESIOLOGY 72:851–857, 1990
17. Shimoji K, Sato Y, Denda S, Takada T, Fukuda S, Hokari T: Slow positive cord dorsum potentials activated by heterosegmental stimuli. Electroenceph Clin Neurophysiol 85:72–80, 1992
18. Davidott RA, Hackman JC: Spinal inhibition, Handbook of the Spinal Cord. Edited by Davidoff RA. New York, Marcel Dekker, 1984, pp 385–459
19. Winer BJ: Statistical Principles and Experimental Design. 2nd edition. New York, McGraw Hill Co., 1962, pp 149–201, 261–308
20. Siegel S: Nonparametric Statistics for the Behavioral Sciences. New York, McGraw Hill Co., 1956, pp 116–126
21. Eccles JC, Schmidt RF, Willis WD: The mode of operation of the synaptic mechanism producing presynaptic inhibition. J Neurophysiol 26:532–538, 1963
22. Eccles JC, Schmidt RF, Willis WD: Pharmacological studies on presynaptic inhibition. J Physiol (Lond) 168:500–530, 1963
23. Schmidt RF: The pharmacology of presynaptic inhibition. Prog Brain Res 12:119–134, 1964
24. Yates BJ, Thompson FJ, Mickle JP: Origin and properties of spinal cord field potentials. Neurosurgery 11:439–450, 1982
25. Rudomin P, Solodkin M, Jiménez I: Synaptic potentials of primary afferent fibers and motoneurons evoked by single intermediate nucleus interneurons in the cat spinal cord. J Neurophysiol 57: 1288–1313, 1987
26. Curtis DR, Lodge D: The depolarization of feline ventral horn group la spinal afferent terminations by GABA. Exp Brain Res 46:215–233, 1982
27. Curtis DR, Gynther BD: Divalent cations reduce depolarization of primary afferent terminations by GABA. Brain Res 422:192–195, 1987
28. Gallagher JP, Higashi H, Nishi S: Characterization and ionic basis of GABA-induced depolarization recorded in vitro from cat primary afferent neurons. J Physiol (Lond) 275:263–282, 1978
29. Akagi H, Yanagisawa M: GABAergic modulation of a substance P-mediated reflex of slow time course in the isolated rat spinal cord. Br J Pharmacol 91:189–197, 1987
30. Kelly MEM, Smith PA: Use of the sucrose-gap technique for quantitative pharmacological studies on isolated adult spinal cord of small mammals. J Pharmacol Methods 19:283–292, 1988
31. Pocock G, Richards CD: Cellular mechanisms in general anaesthesia. Br J Anaesth 66:116–128, 1991
32. Shimoji K, Fujioka H, Ebata T: Anesthetics block excitation with various effects on inhibition in MRF neurons. Brain Res 295: 190–193, 1984
33. Fujiwara N, Higashi H, Nishi S, Shimoji K, Sugita S, Yoshimura M: Changes in spontaneous firing patterns of rat hippocampal neurons induced by volatile anaesthetics. J Physiol (Lond) 402: 155–175, 1988
34. Shimoji K, Fujioka H, Fukazawa T, Hashiba M, Maruyama Y: Anesthetics and excitatory/inhibitory responses of midbrain reticular neurons. ANESTHESIOLOGY 61:151–155, 1984
35. Nicoll RA, Eccles JC, Oshima T, Rubia F: Prolongation of hippocampal inhibitory postsynapic potentials by barbiturates. Nature 258:625–627, 1975
36. Shimoji K, Ito Y, Ohama K, Sawa T, Ikezono E: Presynaptic inhibition in man during anesthesia and sleep. ANESTHESIOLOGY 43:388–391, 1975
37. Jessel TM, Richards CD: Barbiturate potentiation of hippocampal i.p.s.p.s is not mediated by blockade of GABA uptake (abstract). J Physiol (Lond) 269:42p, 1977
38. Hashiguchi T: A new single sucrose-gap method allowing current to be injected and the resulting potential change to be recorded. Pflügers Arch 406:540–543, 1986
39. Hackman JC, Holohean AM, Wohlberg CJ, Davidoff RA: After-hyperpolarizations produced in frog motoneurons by excitatory amino acid analogues. Brain Res 407:94–101, 1987
40. Zhang DX, Hackman JC, Davidoff RA: NMDA antagonists and potentiation of NMDA-induced motoneuron depolarizations in the isolated frog spinal cord. Brain Res 493:129–135, 1989
41. Padjen AL, Mitsoglou GM, Hassessian H: Further evidence in support of taurine as a mediator of synaptic transmission in the frog spinal cord. Brain Res 488:288–296, 1989