J Anesth (1994) 8:321–325

Journal of
Anesthesia

The effect of pentobarbital sodium on the dorsal horn of the spinal cord

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Abstract: The effect of intravenously administered pentobarbital sodium on the activity of single unit in Rexed lamina V of the transected feline lumbar spinal cord was studied using an extracellular microelectrode recording technique. Pentobarbital sodium 1.0 mg·kg^{-1}, 2.5 mg·kg^{-1}, and 5.0 mg·kg^{-1} administered intravenously suppressed both the spontaneous and the evoked activity in Rexed lamina V cells, known to respond principally to noxious stimuli, in a dose-dependent manner. The maximum depression of cell activity occurred within 5 min after intravenous administration. The recovery of cell activity occurred within 70 min after intravenous administration of pentobarbital sodium. We conclude that pentobarbital sodium intravenously administered has a suppressive effect on single unit activity of cells in Rexed lamina V and probably has an analgesic effect. Its suppressive effect is dose-dependent.

Key words: Dorsal horn, Extracellular recording technique, Pentobarbital sodium, Rexed lamina V, Spinal cord

Introduction

Generally, it is believed that barbiturates have no analgesic action [1–4]. Moreover, small doses of barbiturates reportedly increase sensitivity to somatic pain (hyperalgesia or antianalgesic action) [5–9]. However, in clinical use, barbiturates alone are sufficient for general anesthesia for minor short operations such as dilatation and curettage. To investigate the analgesic action of barbiturats, we used spinal cord-transected cats (spinal animal). The spinal cord is a convenient region for testing the effects of anesthetic agents on central connections. Anesthetic agents have been shown to suppress the activities of various aggregates of dorsal horn cells in the feline lumbar spinal cord. Kitahata et al. reported that nitrous oxide [10], ketamine hydrochloride [11], thiamylal sodium [12], and morphine sulfate [13] exert lamina-specific suppression of dorsal horn unit activities. However, there are few reports on the effects of pentobarbital sodium on dorsal horn single cell activity of cells in Rexed lamina V. The purpose of the present study was to investigate the effects of pentobarbital sodium on dorsal horn single cell activity.

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Received for publication on April 8, 1993; accepted on December 21, 1993

Materials and methods

Details of the experimental methods have been reported elsewhere [10]. Procedures specific to the present study are as follows. After approval of the protocol by our institutional Animal Investigation Committee, 30 cats of either sex weighing 2.5–4.2 kg were anesthetized with a mixture of 1.0%–3.0% halothane, 75% nitrous oxide, and oxygen. After tracheostomy and bilateral carotid arterial ligation, the right femoral artery and vein were cannulated. Intravenous infusion of 5% dextrose in lactated Ringer's solution was administered via the femoral vein at a rate of 5–7 ml·kg^{-1}·hr^{-1}. Anesthesia was continued with controlled ventilation using a Harvard ventilater connected to a nonrebreathing system. The cat was placed in a stereotaxic frame and bilateral thermal lesions were made in the midbrain reticular formation. Anesthesia was then discontinued and the animals were ventilated with 100% oxygen, with a tidal volume of 7–10 ml·kg^{-1} and a respiratory frequency of 20–25 min^{-1} to maintain $Paco_2$ at about 30 mmHg. Laminectomy from L1 to S1 was performed. Then the dura was opened, exposing the lumbar spinal cord, which was covered with mineral oil and maintained at 37°C. The spinal cord was transected by electrocautery at the T12-L1 level (Fig. 1). Femoral arterial pressure, pulse rate, and rectal and spinal cord

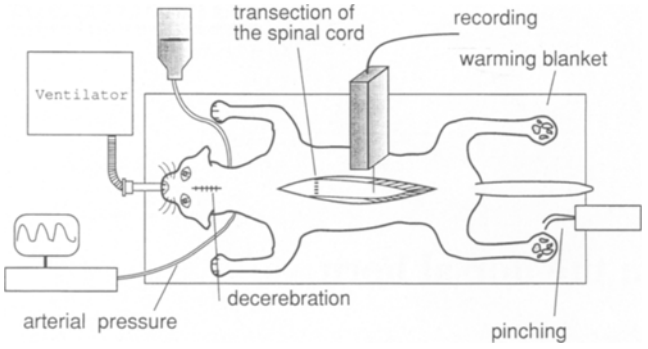

Fig. 1. Experimental apparatus. Tracheostomy, cannulation of the femoral artery and vein, and lumbar laminectomy were done. A cat was decerebrated and the spinal cord was transected between T12-L1. A warming blanket was used to maintain body temperature

temperature were kept within a physiological normal range and recorded continuously on a polygraph. Both rectal and spinal cord temperature were kept constant at 37°C using a heater and infrared lights. Arterial blood gas analyses were performed intermittently. A glass rod platinum-sheathed microelectrode with a 1- to 2-μm exposed tip was then inserted into the lumbar spinal cord near the L7 root entry zone using a hydraulic microdriver. Neurons were characterized by their evoked responses to cutaneous firing patterns [10,14]. A pinching method, using a quantitative pain stimulating (pinching) device (Nihon Kohden, Tokyo, Japan), was used for the investigation of evoked activity. In this study, all physiological characteristics were corrected. Signals were recorded (Sony, WP Instruments, Tokyo, Japan) and simultaneously displayed on an oscilloscope. The spontaneous activity of a single unit was counted electrically, and its instantaneous frequency was displayed on the polygraph. Signals were observed for 15–30 min after isolation to obtain a stable firing pattern and to control the effect of the transient tissue distortion produced by insertion of the microelectrode. After 15 min of the control period, pentobarbital sodium was injected intravenously at doses of 1.0 $mg \cdot kg^{-1}$ in group A ($n = 10$), 2.5 $mg \cdot kg^{-1}$ in group B ($n = 10$), and 5.0 $mg \cdot kg^{-1}$ in group C ($n = 10$). Activity was followed every 5 min until spontaneous activity returned to control values. This required approximately 30–70 min after the administration of pentobarbital sodium. The modality and receptive field characteristics of Rexed lamina V cells recorded were studied before, during, and at the end of recovery from the effects of pentobarbital sodium. For the study of the evoked activity of cells in Rexed lamina V, we used the pinching method, placing an alligator clamp of a quantitative pain stimulating device (Nihon Kohden) in the middle of the receptive field of central nociceptors every 5 min. Paticular care was taken to avoid hypotension and in localization of the electrode tips. Strict controls for physiological status were maintained throughout the experiment. At the end of each experiment, the animals were killed and electrolytic lesions were placed through the recording microelectrode by passing a D.C. current of 20–25 μA for 60 s. The segment of the spinal cord including the microelectrode tract was fixed in situ in 10% formalin. Frozen sections cut at 20–30 μm were stained with cresyl violet. All lesions made were recovered by microscopic observation and correlated with the physiological properties of the units studied. Statistical analysis of the single unit activity stored on the magnetic tape was accomplished off-line using a general-purpose computer (9801RX, NEC, Tokyo, Japan). The mean frequency of cellular activity was obtained during the control period, after administration of pentobarbital sodium, and during the recovery period. The statistical significance of the mean values obtained was assessed by analysis of variance (ANOVA) and Student's t-test. $P < 0.05$ was considered statistically significant. Data are expressed as the mean ± SE.

Results

Blood gas analyses

Results of intermittently analyzed arterial blood gases (pH, Pao_2, $Paco_2$) are shown in Table 1. These results were not significant between each group or between each period.

Effect of pentobarbital sodium on spontaneous single unit activity

The salient features of the physiological characterization of the lamination of the dorsal horn utilized in this study were reported previously [10], and the cells in Rexed lamina V studied responded primarily to high threshold peripheral cutaneous stimuli, deep squeezing, pinching, and the application of high-intensity heat or ethyl chloride. Their spontaneous single unit activity was characterized by bursts followed by steady firing, and their average frequency was 19.4 ± 9.2 I.P.S (impulses per second) (mean ± S.E.). Figure 2 shows a typical polygraph tracing of the firing frequency of unit spikes activity of lamina V before and during the intravenous injection of pentobarbital sodium, and during the recovery period. Figure 3 shows the effect of intravenously administered pentobarbital sodium on spontaneous activity of cells in Rexed lamina V expressed as a percent decrease from the control values over time. A dose-dependent suppression of single unit spontaneous activity of cells in Rexed lamina V by pentobarbital sodium was observed. Five minutes after the adminis-

Table 1. Blood gas analysis data. Results of intermittently analyzed arterial blood gases. No significant differences were seen between groups A, B, and C nor between each period control, immediately after giving pentobarbital sodium and recovery

		Control (mmHg)	After pento. (mmHg)	Recovery (mmHg)
Group A	pH	7.35 ± 0.12	7.40 ± 0.16	7.38 ± 0.21
(n = 10)	Pao_2	368.5 ± 151.3	332.5 ± 132.5	319.0 ± 158.6
	$Paco_2$	38.5 ± 4.6	35.6 ± 3.9	37.5 ± 5.9
Group B	pH	7.41 ± 0.12	7.38 ± 0.21	7.41 ± 0.19
(n = 10)	Pao_2	396.3 ± 162.1	358.3 ± 132.8	360.5 ± 152.3
	Paco2	34.5 ± 4.5	36.3 ± 5.3	38.1 ± 9.2
Group C	pH	7.32 ± 0.13	7.43 ± 0.15	7.37 ± 0.21
(n = 10)	Pao_2	375.8 ± 97.2	360.5 ± 78.7	348.3 ± 169.2
	$Paco_2$	36.7 ± 4.4	37.2 ± 9.2	37.5 ± 13.5

Values are expressed as mean ± SE.
Pento., pentobarbital sodium.

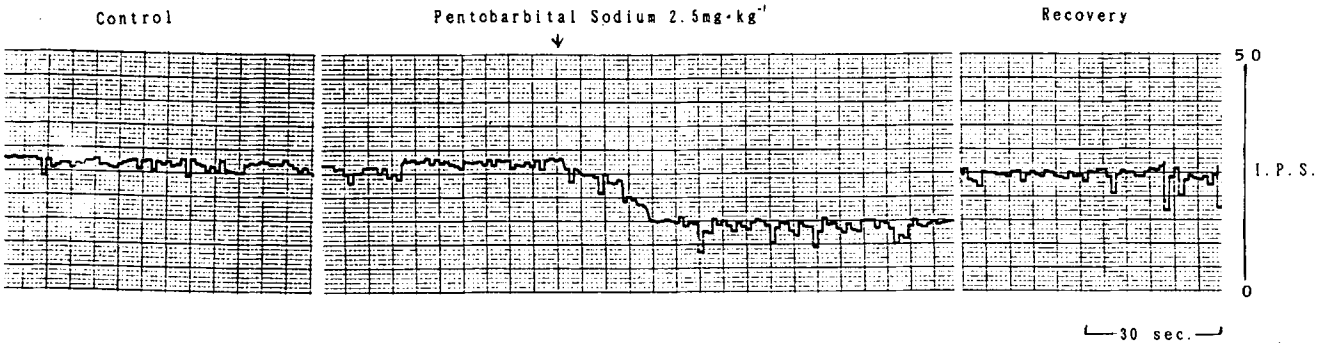

Fig. 2. A polygraph tracing of firing frequency of unit spikes of Rexed lamina V cell. *Left panel*, firing frequency during the control period. *Middle panel*, change of firing frequency induced by intravenous injection of pentobarbital sodium. Firing frequency was significantly decreased by intravenous injection of pentobarbital sodium 2.5 mg·kg^{-1}. *Right panel*, recovery phase. Firing frequency recovered almost to the control level

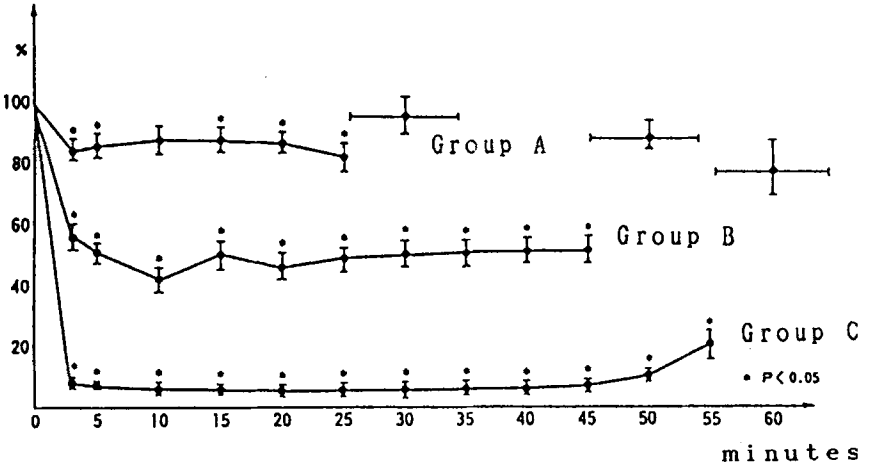

Fig. 3. Effect of pentobarbital sodium on spontaneous lamina V cellular activity expressed as the percent decrease from control levels. Time course after giving pentobarbital sodium 1.0 mg·kg ^{1} (group A), 2.5 mg·kg^{-1} (group B), and 5.0 mg·kg^{-1} (groupC). Immediately after giving pentobarbital sodium, activities were decreased significantly in a dose-dependent manner and recovered to control levels in about 30 min, 50 min, and 60 min, respectively

tration of pentobarbital sodium, the spontaneous firing frequency of cells in Rexed lamina V was significantly suppressed. By analyzing the temporal change of the unit activity of Rexed lamina V cells, we found that spontaneous activity was significantly suppressed 5 min after the administration of pentobarbital sodium and returned to the control level in 30–70 min. The maximum suppression occurred within 5–10 min after administration. The maximum suppression rates were 17.5% ± 4.6% in group A, 57.2% ± 6.9% in group B, and 94.7% ± 5.7% in group C (Table 2). These rates were dose-dependent. These values recovered to the control level in 32 ±5.9 min, 49 ± 10.3 min, and 63 ± 15.4 min, respectively. Figure 4 shows a typical recording of spontaneous active single unit cellular spikes in anatomically verified Rexed lamina V, before, 20 min after administration of pentobarbital sodium 5.0 mg·kg^{-1}, and during the recovery period. As shown in Fig. 3, significant suppression of spontaneous unit activities in a dose-related manner was demonstrated in Rexed lamina V cells.

Table 2. Maximum suppression rates. Both in spontaneous and evoked activity, maximum suppression occurred within 5–10 min after giving pentobarbital sodium. These suppressive rate were dose-dependent in both spontaneous and evoked activity

Group	Group A (%)	Group B (%)	Group C (%)
Spontaneous activity	17.5 ± 4.6	57.2 ± 6.9	94.7 ± 5.7
Evoked activity	12.3 ± 5.8	38.6 ± 7.0	90.5 ± 6.8

Values are expressed as mean ± SE.

Effect of pentobarbital sodium on evoked single unit activity

Evoked activity during the control period was 41.8 ± 13.6 I.P.S. The effect of pentobarbital sodium on the evoked activity of Rexed lamina V is shown in Fig. 5. The maximum suppression after pentobarbital sodium 1.0 mg·kg^{-1}, 2.5 mg·kg^{-1}, and 5.0 mg·kg^{-1}, occurred within 10 min after the administration and were 12.3% ± 5.8%, 38.6% ± 7.0%, and 90.5% ± 6.8%, respectively (Table 2). These suppressions were statistically significant and dose-dependent. These values recovered to control values in 25 ± 6.7 min, 33 ± 6.2 min, and 69 ± 6.3 min, respectively.

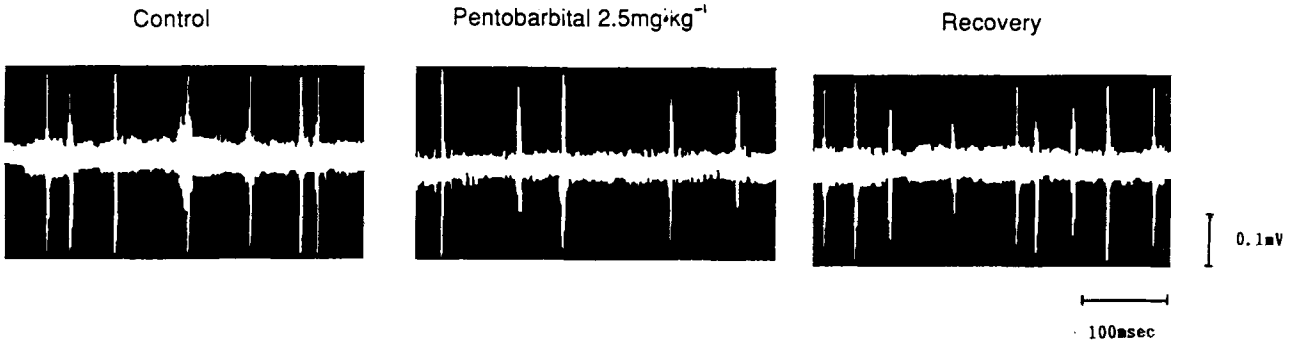

Fig. 4. Spikes of Rexed lamina V cell. *Left panel*, firing during the control period. *Middle panel*, spikes 5 min after pentobarbital sodium. *Right panel*, spikes during the recovery period. Note that firing frequency was diminished by intravenous injection of pentobarbital sodium (*middle panel*)

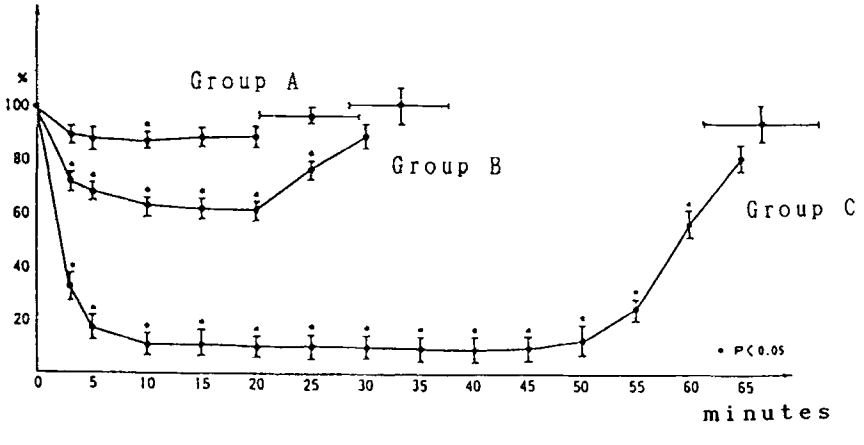

Fig. 5. Effect of pentobarbital sodium on evoked firing lamina V cellular activity expressed as percent decrease from control levels. Time course after giving pentobarbital sodium. As with Fig. 3 (spontaneous activity), pentobarbital sodium suppressed firing frequency in a dose-dependent manner

Discussion

Since the introduction of barbiturates, there has been a controversy as to whether these compounds have an analgesic action. In general, it is accepted that the barbiturates do not have analgesic properties [1–4]. Not only are barbiturates thought to be without analgesic activity at subnarcotic doses, small doses in adults have been observed to antagonize the analgesic action of nitrous oxide [5] or pethidine [6]. In this study, pentobarbital sodium depressed both the spontaneous and evoked activity of cells in the dorsal horn of the feline lumbar spinal cord and the possible effects of the descending supraspinal control mechanism [15] were ruled out by transection of the spinal cord. Variation in excitability of spinal cord cells through variation in ventilation [10] was ruled out by control of ventilation while monitoring $Paco_2$. In correlating the physiologic characteristics of the cells with the anatomical lamination, electrolytic lesions were used. From the results of this study, pentobarbital sodium depressed the spontaneous and evoked activities of cells in Rexed lamina V in a dose-dependent manner. Based on these results, it may be concluded that pentobarbital sodium has an analgesic action on the spinal cord level. Hanaoka et al. reported morphine sulphate suppressed the activity of single unit activity in Rexed Lamina V of the spinal cord-transected cats [16]. They reported a dose-dependent suppression of the activity of lamina V nociceptive neurons after intravenous administration of morphine at doses of 0.5 $mg \cdot kg^{-1}$, 1.0 $mg \cdot kg^{-1}$, and 2.0 $mg \cdot kg^{-1}$. Its suppression rates were 32.5%, 46.0%, and 66.7%, respectively. Comparing our results with these data, the analgesic effect of pentobarbital sodium 1.0 $mg \cdot kg^{-1}$ is less potent that that of morphine sulphate 0.5 $mg \cdot kg^{-1}$ at the spinal cord level. Pentobarbital sodium 2.5 $mg \cdot kg^{-1}$ has roughly the same analgesic effect (equipotent) as morphine sulphate 1.0 $mg \cdot kg^{-1}$, and pentobarbital sodium 5.0 $mg \cdot kg^{-1}$ is more potent than morphine sulphate 2.0 $mg \cdot kg^{-1}$ on the spinal cord level. Lamina V cells in the cat have been shown to receive proprioceptive, high-threshold cutaneous afferent and visceral afferent information [17–19]. Therefore, the depressive effect of pentobarbital sodium on the spontaneous and evoked activities of cells in lamina V may contribute to an analgesic effect on the spinal cord level.

In conclusion, both the spontaneous and the evoked firing frequency of the dorsal horn cells studied were maximally suppressed in a dose-dependent manner 5 to 10 min after the administration of pentobarbital sodium. The spontaneous and evoked firing frequencies of these dorsal horn cells recovered to control level within 30 to 70 min after the administration.

Acknowledgments. This study was supported by a Grant-in-Aid for scientific research (B) No 05454416.

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