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Lamina-specific Suppression of Dorsal-horn Unit Activity by Ketamine Hydrochloride

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The effects of ketamine hydrochloride on single-unit activities of various dorsal-horn Rexed laminae were studied with an extracellular microelectrode recording technique in decerebrate spinal cats. Ketamine hydrochloride, 2.5 mg/kg, iv, suppressed spontaneous single-unit activities of laminae 1 and 5 by 23 and by 43 per cent, respectively, while spontaneous activity of lamina 4 and that of lamina 6 were not significantly affected. This dose suppressed evoked unit activities of laminae 1 and 5 by 44 and by 64 per cent, respectively. As laminae 1 and 5 are known to respond principally to noxious stimuli, a partial explanation for the analgesic effect of ketamine may be lamina-specific suppression of neuronal activity. (Key words: Dorsal horn: Ketamine: Analgesia.)

KETAMINE HYDROCHLORIDE produces profound analgesia and anesthesia in animals [1] and in man.[2, 3] Hypersynchronous delta waves are found in the thalamo-neocortical system,[3, 4] while theta ("arousal") waves are produced in the hippocampus. These findings, "depression" of the thalamo-neocortical system synchronous with limbic "activation," suggested a classification of ketamine as a "dissociative anesthetic."[2-5] This concept was recently challenged by Kayama and Iwama,[6] who concluded that ketamine stimulated the neocortex, hippocampus, and other subcortical nuclei concurrently.

Anesthetic agents have been shown to suppress selectively the activities of various aggregates of dorsal-horn cells in the feline spinal cord. Wall [8] showed that the spontaneous and evoked activities of cells in Rexed [7] laminae 4, 5, and 6 were suppressed by intravenous administration of pentobarbital. Nitrous oxide and halothane were also shown to suppress spontaneous and evoked activity in dorsal-horn cells.[9-11] Lamina-specific supression of dorsal-horn unit activity by nitrous oxide was demonstrated by Kitahata *et al.*,[12] who concluded that the analgesic action of nitrous oxide could, in part, be a result of its selective suppression of lamina 5 cells, which respond principally to noxious stimuli.[13-15]

This study was undertaken to determine whether ketamine acts at spinal cord levels and whether such action is directed specifically toward individual Rexed laminae. Single units whose spontaneous activity and evoked activity were studied were localized with respect to the various Rexed laminae, by physiologic and anatomic criteria. Adequacy of ventilation and circulation, and thermal control, were assured by continuous monitoring of appropriate variables. Strict criteria for relative physiologic normality were adhered to throughout the experiments.[12] Preliminary results were reported previously.[16]

Methods

Forty-six cats of either sex, each weighing 3–4 kg, were anesthetized with a mixture of 2 per cent halothane, 75 per cent nitrous oxide, and oxygen. After tracheostomy and bilateral carotid arterial ligation, the right femoral artery and vein were cannulated. The tip of the arterial cannula was placed just distal to the bifurcation of the internal iliac artery, to avoid compromising the arterial blood supply of the lumbosacral cord. Intravenous infusion of 5 per cent dextrose in half physiologic saline solution with 0.1 per cent gallamine triethiodide was administered via syringe pump at a rate of 5 to 7 ml/kg/hour. Anesthesia was then maintained with controlled respiration

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TABLE 1. Salient Features of Physiologic Lamination of the Dorsal Horn of the Feline Lumbar Spinal Cord (Summary of Characteristics of Rexed Laminae)

	Spontaneous Activity	Receptive Field	"Modality"
Lamina 1	Slow	Large	High-threshold cutaneous; thermal
Lamina 2	None		
Lamina 3	None (brief bursts)	Small	Low-threshold cutaneous
Lamina 4	Bursts and relative silence	Small–large	Low-threshold cutaneous; pressure; pinch; ethyl chloride
Lamina 5	Bursts and steady firing	Large	High-threshold cutaneous; thermal; visceral
Lamina 6	Maintained bursts		Proprioceptive

using a volume-cycled ventilator connected to a nonrebreathing system. The cat was placed in a stereotaxic frame and bilateral thermal lesions were made in the midbrain reticular formation. Electrocorticographic monitoring revealed delta waves only following this procedure and throughout the study. Animals were then ventilated with 100 per cent oxygen, with a tidal volume of 7–10 ml/kg body weight and a respiratory frequency of 20–25/min to maintain end-tidal P_{CO_2} at 34 ± 2 torr. Laminectomy from L1 through S1 was performed. The vertebral column was immobilized with metal clamps. The dura was opened, exposing the lumbar spinal cord, which was then covered with mineral oil kept at 37 C. The spinal cord was transected by electrocautery at L1. Adequacy of spinal cord circulation was gauged by stereomicroscopic observation. Femoral arterial pressure, endotracheal CO_2 level, electrocardiogram, pulse rate, and rectal and spinal cord temperatures were recorded continuously on a polygraph. Both rectal and spinal cord temperatures were kept constant at 37 ± 1 C using a water mattress with a thermal servo-control. Arterial blood–gas analysis was performed intermittently. P_{O_2}, P_{CO_2} and *p*H ranged from 300 to 500 torr, 32 to 36 torr, and 7.30 to 7.45, respectively. A glass-rod platinum-sheathed Transidyne "Microtrode" microelectrode with a 1–2-μm exposed tip was then inserted by a hydraulic micromanipulator into the lumbar spinal cord near the L7 root entry zone. Neurons were characterized by their evoked responses to cutaneous stimulation and by their spontaneous firing patterns.[12] Signals were recorded through a differential FET ac preamplifier on magnetic tape, and were simultaneously monitored on a cathode-ray oscilloscope.

The pulsatile spontaneous activity of isolated single units was counted electronically, and its average frequency displayed on the polygraph. Units were observed for 15 to 30 minutes after isolation to obtain a stable firing pattern and to control the effect of transient tissue distortion by the microelectrode. A 15-minute control period was then recorded. The effect of ketamine upon spontaneous unit activity was then studied by administering 2.5 mg/kg ketamine intravenously. Unit activity was followed until spontaneous activity returned to control values. This took approximately 40 minutes.

The modality and receptive-field characteristics of the cells recorded were studied prior to the administration of ketamine and to the point of recovery of the cell activity from its effects. Lesions were placed via the recording microelectrode with a dc current of 20 to 30 microamperes applied for 10 to 20 seconds.

Placing an alligator clamp in the middle of the receptive field of nociceptors maintained the cellular firing frequency at a constant and elevated rate (higher than the rate without the clamp) for 30–40 minutes. This technique was used to increase background activity in some experiments. Effects of ketamine on the evoked cellular activities of laminae 1 and 5 were studied in eight and in seven cats, respectively.

At the conclusion of the experiment, animals were sacrificed by administration of 100 per cent nitrous oxide. The segment of the spinal cord including the microelectrode tract was fixed *in situ* in 10 per cent formalin for two hours, then removed and fixed in 10 per cent formalin. Frozen sections cut at 20–30

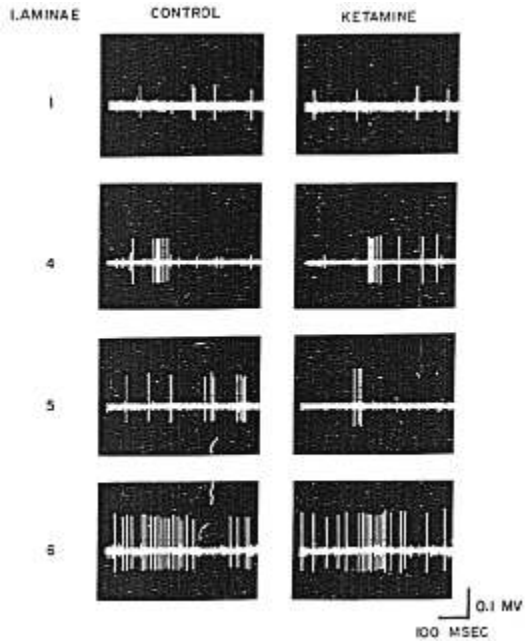

FIG. 1. Single-unit activities of laminae 1, 4, 5, and 6 during the control period and 5 minutes after administration of ketamine hydrochloride, 2.5 mg/kg, iv.

μm were stained with cresyl violet. All lesions made were recovered by microscopic observation and correlated with the physiologic properties of the units studied.

Statistical analysis of the single-unit activity stored on magnetic tape was accomplished off-line using a general-purpose digital computer (DEC PDP12). The mean frequency of cellular activity for runs of 2,048 impulses was obtained during the control period, following administration of ketamine, and during the recovery period. The significance of the means so obtained was assessed by Student's t test.

Results

The salient features of physiologic characterization of the lamination of the dorsal horn utilized in this study were as follows (table 1):

As a microelectrode was passed through the dorsal column, spontaneously active, initially positive, spikes, generally responding to proprioceptive stimuli, were recorded. These were interpreted as primary or secondary afferent fibers. As the electrode was passed further ventrally, the first spontaneous cellular activity was recorded at the level of Rexed lamina 1, slow and steady and responding to high-threshold cutaneous stimuli over a wide field, to extreme deformation of joints, and to thermal stimulation. Laminae 2 and 3 had no spontaneously active cells whose potentials could be isolated from noise with the technique used. In the region of lamina 3, however, brief bursts of single-unit activity could be evoked by hair movement and by light tactile stimuli. Lamina 4 spontaneous activity was characterized by a burst followed by relative silence, the cells responding to low-threshold stimuli, to hair movement as well as to pressure, to pinch, and to application of ethyl chloride. Further ventrally, lamina 5 cells dis-

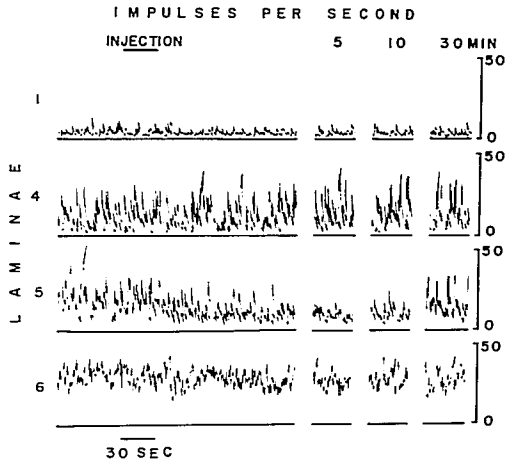

FIG. 2. Unit spike activities of laminae 1, 4, 5, and 6, before and after intravenous injection of ketamine hydrochloride, 2.5 mg/kg.

played the spontaneous activity pattern of a burst followed by steady firing; the cells did not respond to hair movement but generally responded to high-threshold cutaneous stimuli and to proprioceptive stimuli. Lamina 6 manifested greatly increased spontaneous activity in maintained bursts; the cells no longer responded to cutaneous stimuli, but only to proprioceptive stimuli. These overall characteristics of spontaneous activity and gross modalities of responsiveness were highly correlated with the anatomic lamination classification of Rexed.[7]

LAMINA 1 (SIX ANIMALS)

During the control study, while the cats were ventilated with 100 per cent oxygen, the average rate of spontaneous activity was less than 10/sec. The cells responded by increasing their firing frequency only in response to high-threshold cutaneous stimulation, to thermal stimulation, and to extreme deformation of joints, confirming the observations of Christensen and Perl.[17] An example of spontaneous unit spike activity during the control study and 5 minutes following iv administration of ketamine, 2.5 mg/kg, is shown in figure 1. As shown in figure 2, the polygraph display did not show a striking change after administration of ketamine. Statistical analysis, however, demonstrated that ketamine depressed the spontaneous activity of lamina 1 cells by 23 per cent of control values (table 2). Most units studied were sensitive to both mechanical and thermal stimuli,[17] except for two units which were apparently "pure" thermoreceptors, as described by Christensen and Perl.[17] These two units showed no significant effects of the administration of ketamine.

TABLE 2. Effects of Ketamine Hydrochloride (2.5 mg/kg iv) on Single-unit Activity*

	Spontaneous Activity		Evoked Activity	
	Per Cent ± SE	P	Per Cent ± SE	P
Lamina 1	−23 ± 10	<0.01	−44 ± 10	<0.05
Lamina 4	+ 3 ± 1	<0.05		
Lamina 5	−43 ± 10	<0.01	−64 ± 9	<0.01
Lamina 6	+ 3 ± 1	<0.05		

* Percentage changes (− decrease, + increase) compared with control values of spontaneous and evoked single-unit activity of dorsal horn laminae, 5 minutes after iv injection of ketamine hydrochloride, 2.5 mg/kg.

Lamina 4 (Eleven Animals)

The spontaneous activity of lamina 4 cells was characterized by bursts in groups with interspike intervals of 2 to 5 msec with intervening relative silence. The average firing frequency of cells in lamina 4 was 10–20/sec. Figure 1 shows an example of spontaneous unit activity during the control period and 5 minutes following iv administration of ketamine, 2.5 mg/kg. As shown in figure 2, the polygraph display, ketamine did not suppress the spontaneous firing frequency of lamina 4 cells, but, if anything, facilitated their activity. Statistical analysis of the unit activity showed that lamina 4 spontaneous activity 5 minutes after iv administration of ketamine reached 103 per cent of control values (table 2).

Lamina 5 (Seven Animals)

Lamina 5 cells could be identified by a sudden decrease in cellular responsiveness when stimuli were applied to hair. The cells responded only to high-threshold stimuli applied to the receptive field. Their spontaneous activity was characterized by bursts followed by steady firing, with an average frequency of 20–30/sec. An example of spontaneous unit activity during the control period and 5 minutes following iv administration of 2.5 mg/kg ketamine is shown in figure 1. As shown in figure 2, the polygraph display, ketamine markedly suppressed the spontaneous activity of lamina 5 cells. Statistical analysis of the unit activity of lamina 5 cells showed that the spontaneous activity 5 minutes after administration of ketamine was suppressed by 43 per cent of the control values (table 2) and returned to these values 20–40 minutes following the administration of the drug.

Lamina 6 (Seven Animals)

Cells located in the most ventral portion of the dorsal horn were characterized by greatly increased spontaneous activity, in maintained bursts having an average frequency of 20–50/sec. The cells did not respond to cutaneous stimuli, but only to proprioceptive stimuli. An example of the spontaneous unit activity during the control period and 5 minutes following iv administration of ketamine, 2.5 mg/kg, is shown in figure 1. As shown in figure 2, the polygraph display, ketamine had no discernible effect on lamina 6 cellular activity except slight facilitation. Statistical analysis of unit activity of lamina 6 cells showed that their spontaneous activity 5 minutes after administration of ketamine reached 103 per cent of the control values (table 2).

Effect of Ketamine on Evoked Single-unit Activity

High-threshold central mechanoreceptors having slow adaptation could be maintained at a steady firing frequency during constant stimulation of their receptive field. It was found that by placing an alligator clamp over the central part of the receptive field, the activities of cells of laminae 1 and 5 could be maintained at a steady rate for 30–40 minutes. *Lamina 1 (eight animals)*: ketamine, 2.5 mg/kg, iv, suppressed the unit firing frequency by 44 per cent of control values (table 2). Control values returned in 20–40 minutes. *Lamina 5 (seven animals)*: ketamine, 2.5 mg/kg, iv, suppressed the unit firing frequency by 64 per cent of the control values (table 2). Control values returned in 20–40 minuteas.

An example of the laminar localization technique may be seen in figure 3.

Discussion

In this study, effects of ketamine on descending supraspinal[15] control mechanisms were ruled out by spinal section. Variations in excitability through variations in ventilation[19] were ruled out by breath-to-breath monitoring of endotracheal carbon dioxide tension, by control of ventilation, and by direct measurement of blood *p*H, P_{O_2} and P_{CO_2}. Cells within the dorsal horn are exquisitely sensitive to ischemia, and their spontaneous activity may be profoundly suppressed by very brief periods of arterial hypotension.[12] This invariably occurs when animals are studied after high cervical spinal-cord section, and pressor agents or volume expanders are necessary. In this study, high spinal-cord section, pressor agents, and plasma expanders were not used, and unit activity obtained in any experiment after any period of hypotension below 80 torr systolic was rejected from

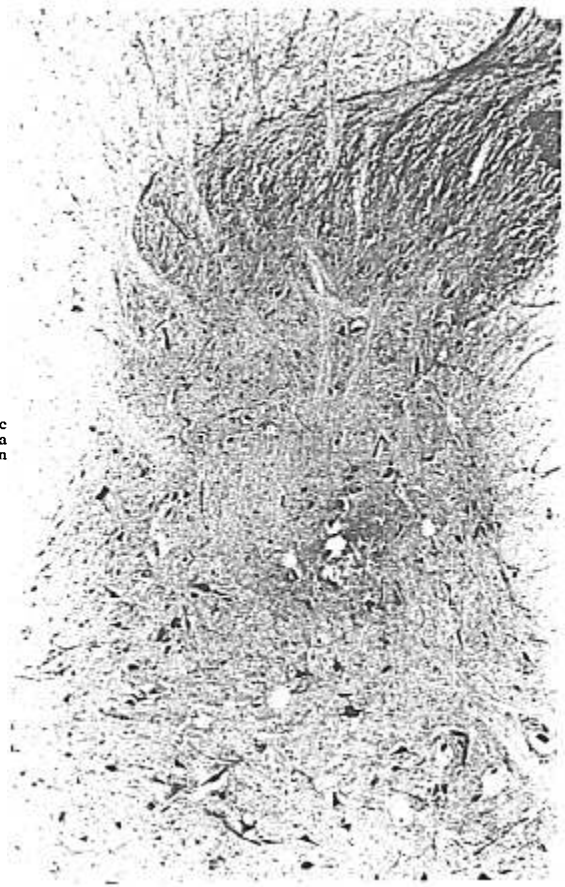

FIG. 3. An electrolytic lesion made through a recording electrode in lamina 5.

the analysis. This occurred only rarely, however.

In order to correlate physiologic characteristics with anatomic structures, the resolving powers of the techniques used must be comparable. A cleared, freehand-section, microelectrode-*in-situ* technique, as utilized by others,[6, 12] can provide only an approximation, and because of obliquity of section and individual variation, can mismatch physiologic and anatomic information. Electrolytic lesions, as they are used here, are destructive and reveal only remaining cells, yet they do localize, as between laminae.

The results of this study demonstrate that ketamine, known to have profound analgesic

effects clinically, selectively suppresses spontaneous and evoked activity of lamina 5 of the feline dorsal horn. Lamina 5 has been shown to receive proprioceptive and high-threshold cutaneous afferent and visceral afferent information.[12-15] The selective suppression of a dorsal-horn lamina responding to high-threshold cutaneous and visceral afferents may contribute in part to the analgesic effects of ketamine at the spinal level.

Lamina 1 cells have been demonstrated to respond only to high-threshold cutaneous and thermal stimulation and to receive input from high-threshold cutaneous A-delta fibers.[17] In the present study, statistical analysis of unit activity revealed that spontaneous and evoked activity of lamina 1 cells was suppressed by ketamine. The analgesic action of ketamine may be explained in part through its suppressive action on lamina 1 cellular activity as well as on lamina 5 cellular activity.

The data further show that the spontaneous unit activity of lamina 4 and that of lamina 6 are essentially uninfluenced or are slightly accelerated.

Conseiller *et al.*[20] obtained similar results in a recent study of the effects of ketamine upon dorsal-horn unit activity. In their study, lamina 4 cells were unaffected, while the spontaneous activity of lamina 5 cells showed a "slight decrease," in contrast to the effects produced upon the activity resulting from noxious stimulation, where decreases in unit activity approached 25 per cent. Quantitative differences between the findings of Conseiller *et al.*[20] and the data presented here might be accounted for by differences in technique, inasmuch as Conseiller *et al.* utilized C1-spinal-cord-sectioned animals, localized electrodes by a freehand, cleared-section method, gave a dose of ketamine four times greater (10 mg/kg) than that used in this study, and did not use statistical techniques to evaluate their results.

Why ketamine suppresses the activity of one group of cells while facilitating that of another group is not known. This differential effect of ketamine, however, is not unique, as nitrous oxide has similar effects on the various dorsal-horn laminae.[12]

While the experimental data presented here clearly indicate a marked suppressive effect of ketamine upon the spontaneous and evoked activities of laminae 1 and 5, they do not indicate the mechanism of such suppression, which remains to be determined.

Also, since ketamine has been demonstrated to produce striking supraspinal effects,[2-6] it remains to be seen whether the effects demonstrated here to exist at the spinal level are causally related to those at higher levels, or are independent. The simplest hypothesis at this time would be to suppose that a portion of the analgesic effect of ketamine is determined at the spinal level by the differential suppression of the activity of cells in dorsal-horn laminae responding to noxious peripheral stimuli.

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Metabolism

CATECHOLAMINE EXCRETION IN CYANOTIC CONGENITAL HEART DISEASE Urinary catecholamines were studied in 23 normal children 13 days to 14 years of age and 25 children with cyanotic heart disease 3 days to 10 years of age.

Significant elevations of dopamine (DA) and combined metanephrine and normetanephrine (MN + NMN) were found in the cyanotic group. Epinephrine (E), norepinephrine (NE) and 3-methoxy-4-hydroxymandelic acid (VMA) values showed no significant differences, but the cyanotic children tended to have higher values than normal. The increase of the precursor DA and of the principal metabolites MN + NMN plus the tendency to high urinary levels of E and NE make it probable that the cyanotic children had increased endogenous secretion of E and NE.

The authors speculate regarding the significance of the increases of E and NE in the cyanotic child: 1) enhancement of positive inotropic myocardial effect; in individuals with outflow tract obstruction from either ventricle this may result in an even larger reduction of pulmonary blood flow and an increase in hypoxemia; 2) increased tissue oxygen consumption; in the presence of oxygen deprivation the resulting metabolic acidemia may be the single most important element in the morbidity and mortality of the neonate. (*Folger, G. M., Jr., and Hollowell, J. G.: Excretion of Catecholamine in Urine by Infants and Children with Cyanotic Congenital Heart Disease, Pediat. Res. 6: 151–157, 1972.*)