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# Effects of Althesin, Sodium Thiamylal and Diazepam on Single Unit Activity of Various Dorsal Horn Rexed Laminae

(spinal cord/Althesin/sodium/thiamylal/diazepam/anesthesia)

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**Effects of Althesin, sodium thiamylal and diazepam on single unit activities of various dorsal horn Rexed laminae were studied with an extracellular microelectrode recording technique and using decerebrate spinal cats. Althesin 100 μl/kg I. V. suppressed spontaneous single unit activities of lamina 1, 4, 5 and 6 by 60, 48, 51 and 38%, respectively at 2–3 min after injection. Sodium thiamylal 5 mg/kg I. V. suppressed spontaneous single unit activities of lamina 1, 4. 5 and 6 by 42, 49, 70 and 34%, respectively. Diazepam 0.5 mg/kg suppressed spontaneous single unit activities of lamina 1, 4, 5 and 6 by 38, 72, 66 and 48%, respectively.**

**The effects of Althesin 100 μl/kg on single unit activities of dorsal horn cells are compared with those of sodium thiamylal 5 mg/kg and diazepam 0.5 mg/kg. These drugs suppressed the activities remarkably in all Rexed laminae at 2 to 5 min. A lamina specific suppression, such as was seen in the case of ketamine hydrochloride and morphine sulfate, was not apparent. The degree of the suppression after administration of diazepam 0.5 mg/kg I. V. was the largest among these drugs and recovery from the effects required the longest time. The recovery from the effect was the most rapid in the case of Althesin. There were, however, no other significant changes among these drugs.**

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In 1952, Rexed (1) reported the cytoarchitectonic organization of the spinal cord of the cat. Subsequently, Melzack and Wall (1965) (2) postulated the gate control theory in which the substantia gelatinosa in the spinal dorsal horn was involved in the organization of mechanisms related to pain. With the acceptance of this theory, a number of workers studied the dorsal horn cells. The effects of anesthetics on the unit activity of dorsal horn cells have been given little attention.

We have shown that ketamine hydrochloride (3), morphine sulfate (4), halothane and thiopental (5) exert lamina specific suppression of dorsal horn unit activities and we concluded that a portion of the analgesic action of these agents is determined at the spinal level by differential suppression in dorsal horn lamina 1 and 5 of the activities of the cells which respond primarily to noxious peripheral stimuli (4).

The present study was undertaken to determine whether clinical doses of Althesin (6,7), thiamylal and diazepam act at spinal cord levels and whether such actions are directed specifically toward individual Rexed laminae. Adequacy of ventilation and circulation and thermal control were assured by continuous monitoring of appropriate variables. Preliminary results were reported previously (8).

## MATERIALS AND METHODS

Fifty-one cats of either sex each weighing 2.5—4 kg, were anesthetized with a mixture of 2% halothane, 75% nitrous oxide and oxygen. After tracheostomy and bilateral carotid arterial ligation, the right femoral artery and vein were cannulated. Lactated Ringer's solution with 0.1% gallamine triethiodide was infused via a syringe pump at a rate of 5 to 7 ml/kg/hour. Anesthesia was then maintained with controlled respiration using a volume cycled ventilator connected to non-rebreathing system. The cat was placed in a stereotaxic frame and bilateral thermal lesions were made in the midbrain reticular formation. The cats were then ventilated with 100% oxygen, with a tidal volume of 7—10 ml/kg body weight and a respiratory frequency of 20—25/min to maintain end tidal $Pco_2$ at 34±2 torr. A laminectomy was carried out from $L_1$ through $S_1$ and metal clamps were used for the immobilization. The dura was opened and the exposed lumbar spinal cord was covered with mineral oil and kept at 37°C. The spinal cord was transected by electro cautery at $L_1$. Adequacy of spinal cord circulation was gauged by stereomicroscopic observation. Both rectal and spinal cord temperatures were kept constant at 37±1°C using a water mattress with a thermal servocontrol. Arterial blood gas analysis was performed intermittently. $Po_2$, $Pco_2$ and pH ranged from 300 to 500 torr, 32 to 36 torr and 7.30 to 7.45, respectively. A glass rod platinum sheathed Transidyne "Microtrode" micromanipulator was inserted into the lumbar spinal cord near the $L_7$ root entry zone. Neurons were characterized by their evoked responses to cutaneous stimulation and by their spontaneous firing patterns. (3, 9). Signals were recorded on magnetic tape and were simultaneously monitored on a cathode-ray oscilloscope. The pulsatile spontaneous activity of isolated single unit was counted electronically. Units were observed for 15 to 30 min after isolation to obtain a stable firing pattern and to control the effect of transient tissue distortion by the microelectrode. A 15 min control period was then recorded. The effect of drugs upon spontaneous unit activity was assessed.

## RESULTS

The salient features of physiologic characterization of the lamination of the dorsal horn in the feline spinal cord were as previously reported (3,4). These characteristics of spontaneous activity and gross modalities of responsiveness were highly correlated with the anatomic lamination classification of Rexed.

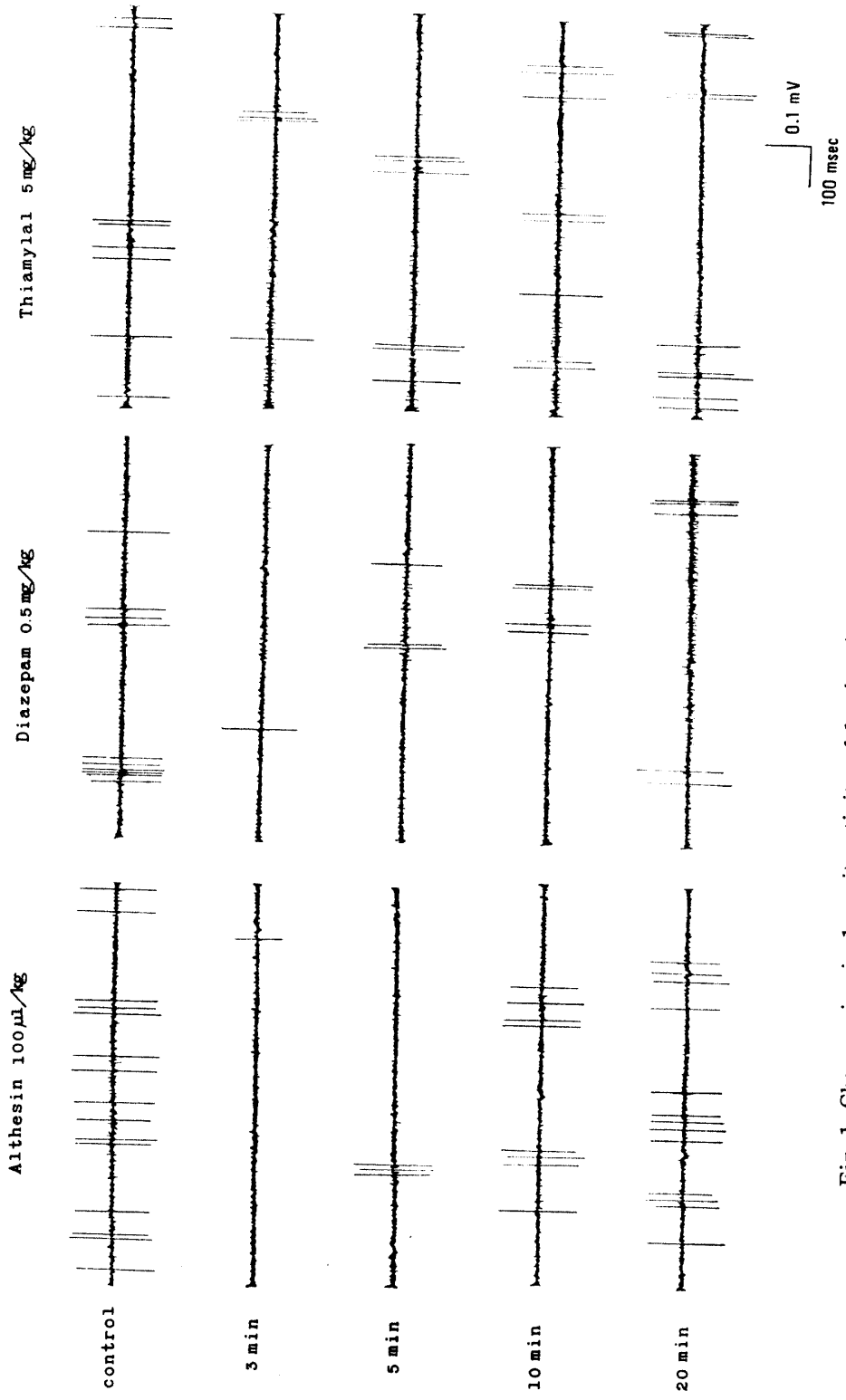


Fig. 1. Changes in single unit activity of lamina 4 cells of cats, after administration of Althesin 100 $\mu$l/kg, diazepam 0.5 mg/kg and sodium thiamylal 5 mg/kg.

Fig. 1 shows the change of single unit activity of lamina 4 cells, after administration of Althesin, sodium thiamylal and diazepam.

The average firing frequency of cells in the dorsal horn utilized were 5—9/sec (lamina 1), 15—25/sec (lamina 4), 14—30/sec (lamina 5) and 19—30/sec (lamina 6), respectively.

*Effects of Althesin*

Fig.2 shows the change in the spontaneous activity of the cat spinal cord

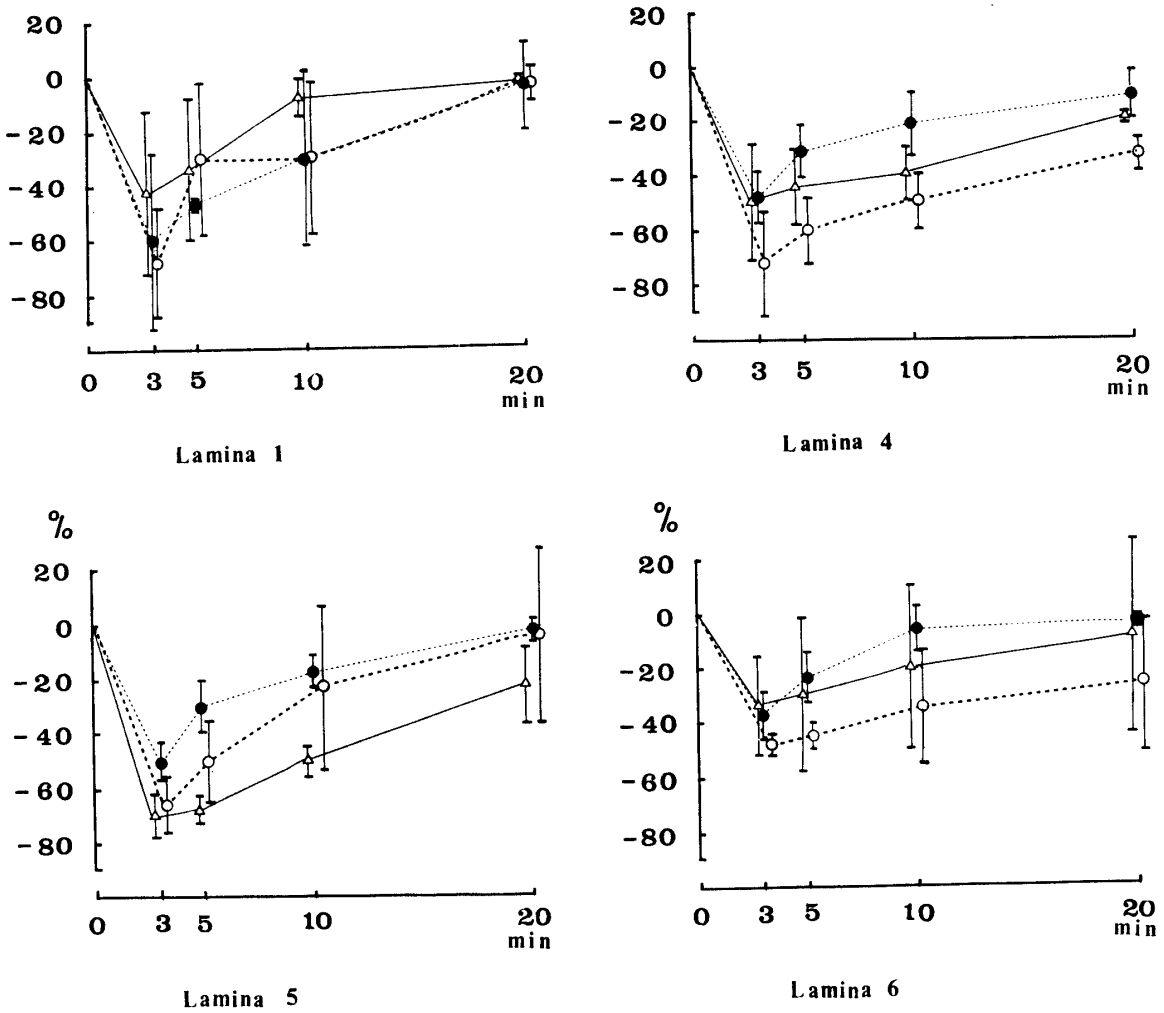


Fig. 2. Changes in the spontaneous activity of Rexed laminae in cats, after Althesin 100 μl/kg, diazepam 0.5 mg/kg and sodium thiamylal 5 mg/kg I. V., respectively. ●·····● : Althesin 100 μl/kg, ○·····○ : Diazepam 0.5mg/kg, △——△ : Thiamylal 5mg/kg.

cell, after administration of Althesin 100 μl/kg infusion via the femoral vein over a 30 sec period. At 2—3 min after this administration, the spontaneous activity was markedly suppressed in lamina 1 by 60%, lamina 4 by 48%, lamina 5 by 51% and lamina 6 by 38%. Ten min after the injection, a remarkable recovery was seen and after 20 min the recovery was all but complete. Althesin 100 μl/kg I. V. suppressed lamina 6 to a lesser extent than the other laminae.

*Effects of Sodium Thiamylal*

Changes in the spontaneous activity of spinal cord cells after sodium thiamylal 5 mg/kg I. V. over a 30 sec period are shown in Fig. 2. This dose of sodium thiamylal suppressed maximally the spontaneous single unit activities of lamina 1, 4, 5 and 6 by 42, 49, 70 and 34%, respectively. Lamina 5 was markedly suppressed and lamina 6 was the least suppressed. At 20 min after the injection, all except lamina 5, had recovered to some extent from the suppression.

*Effects of Diazepam*

Fig. 2 shows changes in the spontaneous activity of spinal cord cells, after 0.5 mg/kg was infused through the femoral vein over a 30 sec period. At 2—3 min after this injection, the laminae were suppressed most markedly, and 10 min later, this suppression diminished somewhat. After 20 min, the suppression in lamina 1 and lamina 5 recovered completely, but the suppression in lamina 4 and lamina 6 continued. This suggests that a small dose of diazepam exerts specific suppression in lamina 4 and lamina 6.

*Comparison of Effects on Dorsal Horn Cells*

Fig. 2 represents a graph indicating the comparison of the change of unit activity after administration of these drugs. Althesin, sodium thiamylal and diazepam all markedly suppressed single unit activities at 2—3 min after administration.

TABLE I. *Relationship between the Degree of the Suppression and the Recovery from Althesin 100 μl/kg (A), Diazepam 0.5 mg/kg (D) and Sodium Thiamylal 5 mg/kg (T) I. V., respectively*

| | Suppression | Recovery |
|---|---|---|
| Lamina 1 | D>A>T | T>A≒D |
| Lamina 4 | D>T≒A | A>T>D |
| Lamina 5 | T≒D>A | A>D>T |
| Lamina 6 | D>A=T | A>T>D |

Table I shows the relationship of the degree of the suppression and the recovery from Althesin 100 μl/kg I. V., diazepam 0.5 mg/kg I. V. and sodium thiamylal 5 mg/kg I. V., respectively. The degree of the suppression after administration of diazepam 0.5 mg/kg I. V. was the largest among these drugs, the recovery from the effects was the latest. The recovery from the effect was the most rapid in the case of Althesin. The recovery from the effect of sodium thiamylal in lamina 1 and lamina 6 was faster than seen with the other laminae. The suppression of unit activities of lamina 4 and lamina 5 by sodium thiamylal, and of lamina 4 and lamina 6 by diazepam continued, even at 20 min after administration.

## DISCUSSION

Wall (10) showed that the spontaneous and evoked activities of cells in

Rexed lamina 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 (11,12). Lamina specific suppression of dorsal horn unit activity has been demonstrated by Kitahata *et al.* using nitrous oxide (9), ketamine hydrochloride (3) and morphine sulfate (4). These agents have primarily an analgesic action and a lesser hypnotic effect. These same workers demonstrated that sodium thiopental 2.5 mg/kg, 5 mg/kg and 10 mg/kg I. V. given to decerebrate, unanesthetized and spinalized cats have a significant dose dependent suppressive action, not only on the activity of cells responding primarily to nociceptive stimuli (cells in Rexed laminae 1 and 5), but also on the activity of cells in lamina 4, with proprioceptive input (5). They reported that sodium thiopental 5 mg/kg I. V. suppresed spontaneous single unit activities of laminae 4, 5 and 6, by about 8%, 60% and 17%, respectively, at 5 min after infection.

The difference between the effects of thiopental and thiamylal is so slight, that such can be detected only by elaborate statistical analysis (13). In a comparative clinical study of their actions Tovell and colleagues (14) were unable to find any difference between the two drugs, and Gilmore and Dundee (13) found in a "blind" study that they could not be distinguished.

There is a very large dosage range (40 to 150 $\mu$l/kg) in which Althesin is acceptable as an induction agent (7). Pickerodt and colleagues (15, 16) reported that Althesin, markedly reduces cerebral oxygen uptake and that the metabolic depression leads to a secondary fall in cerebral blood flow and cerebrospinal fluid pressure. Clarke *et al.* (17) estimated that 60 $\mu$l/kg Althesin appears to be equipotent with 4 mg/kg of thiopental. The one detailed study shows that Althesin is shorter-acting than equivalent doses of thiopental, but longer than that of methohexitone (7). In our study, the recovery from the effect was the most rapid in the case of Althesin.

Ngai *et al.* confirmed that diazepam acts primarily upon supraspinal structures, most likely on the reticular facilitatory system (18). The central areas most likely concerned with the effects of diazepam are the limbic system, consisting of the hippocampus, the amygdala, the thalamus, the fornix and the cingulate gyrus (7). One of the principal effects of diazepam is its action on the skeletal muscle. The reduction of muscle rigidity in decerebrate cats places the level of action of diazepam at the spinal cord, and since the monosynaptic knee jerk reflex is not impaired, it is deduced that diazepam interferes specifically with interneuronal transmission. The neuromuscular blocking action of diazepam is difficult to evaluate because of the degree of muscle relaxation resulting from its action on the spinal cord and reflexes (7). Clinically there is a great individual variation in response to diazepam. In some patients a very small dose will produce an unconscious state while others may only be drowsy after 1 mg/kg. From the data presented by Brown and Dundee the anesthetic dose varies from about 0.25 to 1.5 mg/kg (19). The recovery from diazepam if very slow and even after small doses of 0.1 to 0.3 mg/kg recovery is frequently slow (20). In our experiment, recovery from the effect

of diazepam was very slow.

We found that the activities in all laminae were markedly suppressed by Althesin and diazepam I. V., but a lamina specific suppression such as was seen in the case of ketamine hydrochloride and morphine sulfate was not apparent. Such may explain the relatively large doses of these drugs used in our study.

Dose response curves of each drug are now being investigated in our laboratory.

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