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Some Pharmacological And Histological Studies On The Effect Of Neostigmine Injected Intrathecally

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Abstract

The spinal delivery of the cholinesterase inhibitor neostigmine yields analgesia and augments the analgesic effect of alpha-2 (α-2) agonist.

To assess its activity, histological and pharmacological studies were designed to define its effect in two species; rats and cats.

Pharmacological assessment of intrathecally injected (it) neostigmine in cats showed a gradual increase in mean blood pressure (MBP) and heart rate (HR) at doses 2 – 16 μg /kg while a decrease in MBP and HR occurred at doses 32 – 64 μg /kg. The intrathecal injection of atropine and phentolamine abolished the increase in MBP and HR produced by (it) neostigmine (4 μg /kg). In spinal cat preparation (it) neostigmine produced rapid rise in MBP at small (4 μg /kg) and large doses (64 μg /kg). In this study neostigmine counteracts the hypotensive effect and bradycardia produced by intrathecal injection of α-2 agonist clonidine.

Histological study was performed on rats. They were divided into 5 groups representing control and 4 groups treated by neostigmine at 25, 50, 75 and 100 μg/kg. After each injection, the animals were assessed for general behavior, and function. Arousal, motor coordination and motor tone measurement (A, MC and MT) revealed that Intrathecal injection of neostigmine resulted in dose-dependent decreased arousal, and motor coordination, and dose-dependent increase in motor tone.

The quantitative histological and cytochemical data demonstrated an initial increase in the nucleo-cytoplasmic ratio of the anterior horn cells up to 75 ug/kg followed by a decline in 100 ug/kg- treated animals. The cytoplasmic RNA content of the anterior horn cells showed an increase in the optical density that reached a maximum at 50 ug/kg followed by a decline at higher doses. The Golgi bodies increased in the cytoplasm of 25 ug/kg treated animals, the level became constant up til 75 ug/kg, and started to decline at 100 ug/kg. There was no change in the quantity of the myelinated nerve fibers, however, there was a dose dependent decline in their stainability with silver.

In conclusion:These results provide an evidence that the adverse events from neostigmine injected intrathecally appear to be affected by the dose injected which could be important in clinical practice

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Introduction

Surgical trauma is a noxious stimulus of the body that produces a range of biologic alterations. The choice of anesthesia and postoperative pain reliever may have an important implication for postoperative patient outcome (14).

Intrathecal injection of neostig - mine represents a cholinergic mechan - ism of spinal analgesia. It inhibits the breakdown of the endogenous neurot - ransmitter- acetycholine- which has been shown to cause analgesia(2) Also it potentiates analgesia from intrath - ecally administered α-2 adrenergic agonists in rats and sheep (7).

Clinical assessment in humans showed that intrathecal neostigmine causes dose dependent analgesia and side effects (nausea, vomiting, weakness, sedation), the degree of which depends on the amount of tonic release of acetylcholine (13).

Clinical trials of any new agent or new route of administration are gener - ally performed using an open label, dose escalating design in healthy patients or volunteers (15) . The focus of these studies is to assess the safety and estimate the relationship between dose and incidence of side effects. Laboratory studies demonstrated a dense binding of cholinergic ligands in the superficial dorsal horn; and microinjection of cholinergic agonists in this area inhibits excitation of dorsal horn neurons by electrical stimulations. However, the clinical utility of intrath - ecally administered cholinergic agonist, neostigmine, may be limited by motor weakness caused by direct stimulation in the spinal cord ventral horn (21).

The aim of the present study is to evaluate the effect of intrathecal injection of neostigmine on the morphology and histochemistry of anterior horn cells in rats in which behavioral changes are also well observed. The phamacological effects of intrathecal neostigmine, either alone, or in combination with clonidine on the cardiovascular variables in cats are also tested in thus study.

MATERIAL AND Methods

Drugs

Neostigmine methylsulphate : Eipistgmin vial (2.5 mg/ml , Epico)

Clonidine hydrochloride : Catapresan ampoule (150 mg/ml), Boehringer Ingelheim .

Atropine sulphate : ampoule (1 mg/ml)Cid

Epinephrine : ampoule (1mg/ml). (Misr)

Phentolaminc :Regitine ampoule (10 mg/ml)(Novartis)

Doses: therapeutic doses were converted to the animal dose according to surface area (19).

Injection protocol

Dilution of each drug was made with an aseptic technique using sterile saline (0.9% NaCl). In rats, the drug was delivered in a volume of 10 µl, while in cats it was delivered in a volume of 1 ml.

After each injection, a second 10 µl or 1 ml of sterile saline was injected to flush the needle in each animal respectively.

The study was divided into two parts.

Part I: Pharmacological studies

In Vivo studies

1-Experiments on arterial blood pressure and electrocardiograph of anesthetized cats (11).

Cats of both sexes, weighing 2-3kg, were anesthetized by intraperitoneal injection of pentobarbitone (20 mg/ kg).

The femoral artery was exposed, cannulated and the cannula was conne - cted to a mercury manometer provided with a lever writing on moving smoked

kymograph drum. Electrocardiograph (ECG) was recorded throughout the experiment using the standard lead (lead 11) speed record 25mm/ sec (Siemens model cardiostat). The mean blood pressure (MBP) (diastolic blood pressure +1/3 pulse pressure) and ECG were simultaneously recorded.

The following experiments were done:

- The effect of IT neostigmine (2 - 64µg/kg) on MBP and ECG of anesthetized cats was recorded.
- The effect of IT neostigmine on MBP of anesthetized cats was examined before and after IT injection of the muscarinic antagonist, atropine (500 µg/kg); and the noradrenergic antagonist, phentolamine (8 µg/kg).
- The interaction between IT neostigmine and clonidine (25 µg/kg**)** on MBP of anesthetized cats was shown by giving each drug alone and when IT clonidine was injected 60 minutes after IT neostigmine.

2- Spinal cat Preparations:

The effect of IT neostigmine in small dose (4 µg/kg) and in large dose (64 µg/kg) was examined on MBP of spinal cat preparation.The anesthetized animal was placed on its back and both carotid arteries were tied as high as possible in the neck. The animal was then placed on its abdomen and the head was flexed forward. Care being taken to see that the tracheal cannula and trachea were not kinked. A longitudinal cut was made in the middle line of the skull down to the back of the neck. The skin and the muscle layers were retracted, the occipital bone at the back of the skull and the first cervical vertebra were exposed. The cisterna magna was felt between the two bones as a fluid –filled mass; when this was cleaned, it became transparent and the underlying spinal cord was seen. The animal was connected to the respiratory pump. The cisterna magna was opened and the spinal cord was cut with a thick blunt edged probe about 5 mm width, by 1-2 mm thickness.A special rod, maximum diameter 5 mm was pushed into the brain through the opening at the base of the skull, the foramen magnum, and stirred around in order to destroy the brain.

Part II : Histological study

Repeated fixed- dosing.

The test was conducted on 30 rats (200 g each), they were anesthe - tized with ether. The surgical field on the dorsum of the lumbosacral region was prepared with betadine and alcohol.

A skin incision was made and muscles were retracted to expose the vertebral column. A fine needle was inserted into the lumber inter-vertebral space, fixed and secured. On anesthetic recovery, if the rat showed a deficit on hindlimb function it was excluded from the study.

The rats were divided into five groups (n=6 each group). Intrathecal (it) neostigmine was injected in doses of 25, 50, 75 and 100µg/kg body weight which were equivalent to 250 µg, 500µg,750 µg and 1mg intrathecal neostigmine human dose. The rats were assigned to one of the following treatments:

(It) saline (control group) (n=6) , (it) neostigmine (25µg/kg), (it) neosti - gmine (50µg/kg), (it) neostigmine (75 µg/kg) and (it) neostigmine (100µg/kg). Each group received bolus injections for four repeated doses, 30 minutes apart. After each injection the animals were assessed for general behavior and function as described in table (1) . Tremors and salivation were also assessed. The time of each inject - ion was arranged to coincide with the

time of peak concentration of neostig - mine in the cerebrospinal fluid after the previous injection of this drug. This time ranged from 5-30 min (21,22).

Rat Sacrifice

On the second day, the animals were sacrificed and the vertebral column from the lower sacral to the cervical area was dissected out. Excess tissue was removed leaving the vertebral column intact. It was fixed in neutral buffered formol saline (NBFS) for at least one week. The spinal cord at the level of cervical region was dissected out; cut and 0.5 mm long specimen were fixed in NBFS for 48 hours. The tissues were processed for paraffin embedding and blocking. Thin (6 μm) sections were mounted on clean microscopic glass slides and stained with the following staining techniques:
Hematoxylene and Eosin stain (H,E stain) (8).
toluidine blue for Nissle granules (8).
Methyl green pyronin for RNA and DNA (6) .
Nauta and Gygax silver for nerve fibres (18) .

Quantitative analysis :-

The following parameters were measured using computerized micros - copic image analyser with Optimas software version 6.21 (Media Cybernetics Inc.). Two slides from each animal were used to obtain data representing each of the following parameters :-

1-Nucleo -Cytoplasmic ratio (NCR):

The volume of nerve cell nuclei (NV), and the volume of the cells (CV) was measured. The NCR was calculated from the following equation:

NCR = (NV x 100/ CV)

2- RNA content:

The cell content of RNA was measured in nerve cells stained with pyronin. The content was expressed as optical density values according to the Lambert law (26).

3- Golgi body content:

The Golgi bodies were evaluated as the intracellular, non-nuclear, silver stained material.

4- Nerve fiber evaluation:

The volume of nerve fibers was evaluated as the area occupied by the fibers stained by silver in a fixed area box and sections of the same thickness. The change in myelin properties was measured as the optical density (concentration) of stained myelin.

Data were collected and analysed using student's test. Results were expressed as mean ± standard deviation $P<0.05$and $P<0.001$were considered statistically significant.

Results

Part I: Pharmacological Studies

Intrathecal injection (it) of neos - tigmine in small doses (2 -16 μg / kg) produced a dose dependent increase of mean arterial blood pressure (MBP) and heart rate (HR) of anesthetized cats. This effect appeared 15min after injection and increased gradually until it reached the maximum effect at 60 miutes. The increase in MBP was statistically significant while changes in heart rate were insignificant (Figure 1,2 , table 2).

The muscarinic antagonist, atropine, injected intra-thecally at dose 500μg /kg abolished the increase in MBP produced by (it) neostigmine at 4μg/kg (Figure 3). Also, the adrenergic antagonist, phentolamine, injected intrat -hecally at a dose of 8μg/kg abolished the increase of MBP produced by it neostigmine at 4μg/kg (Figure 4).

However, (it) neostigmine in large doses (32-64 μg/kg), produced a decrease of MBP of anesthetized cats (figure 5), which was abolished by (it) atropine (Fig.6).

After destruction of the brain in spinal cat preparation, it neostigmine in small doses (4μg/kg) and in large doses

(64µg/kg) produced a rapid rise of MBP (Figures 7,8).

Although (it) neostigmine, at 4µg/kg, produced an increase in MBP and HR of anesthetized cats; (it) clonidine (25µg/kg) decreased both HR and MBP in these animals. When neostigmine was injected intrathecaly, at a dose of 4µg/kg, 60 minutes before clonidine, it counteracted the hypotension and bradycardia produced by clonidine (Figure 9,10).

Part II: Histological studies

Arousal Motor coordination and Motor tone measurement (AMC and MI):

The intrathecal injection of neostigmine resulted in decreased arousal which was dose dependent (figure 11). This was evidenced by decreased activity with increasing dose to no organized response to pinch of the forepaw.

Decreased motor coordination was also observed by increasing the dose of neostgmine (figure 12). The rats ambulated asymmetrically. This became extreme at the 2nd dose of 75µg/kg neostigmine, while the rats could not functionally move in a forward fashion with the 3rd and 4th injections of the same dose. This effect was observed with 100 µg/kg neostigmine from the start of drug injection and throughout the study period.

Increasing the dose of intrath - ecally injected neostigmine resulted in an increase in motor tone (figure 13). This was evidenced by increasing stiffness of the chest wall with extension of limbs. Tremors were observed when the paw was gently withdrawn. Convulsions were seen 5min after each intrathecal injection of 100µg/kg neostigmine.

Comparing the four doses injected intrathecally, tremors were observed with injection of 100 > 75 >50 >25 µg/kg. The onset of increased tone was observed 5min after injection. Like motor tone, salivation was observed 5min after injection of each of the 75 and 100 µg/kg. Salivation was profuse with 100 µg/kg neostigmine throughout the study period. Also, intrathecal injection of 100 µg/kg neostigmine resulted in diarrhea and protrusion of the eye throughout this study.

Structural and cytochemical study

The general structure of the ventral column cells and fibers of control and treated animal spinal cord shows no clear cut changes (plate 1). However, the nucleo cytoplasmic ratio of the nerve cells, which is one of the parameters used to indicate nuclear activity, is different in treated animals compared to control (figure 14). The ratio increases as the dose increases up to 75 µg./kg of intrathecal neostigmine injection, then decreased to a level higher than control in animals injected with 100 µg / kg.

The cytoplasmic content of Nissl granules (plate 2) and cytoplasmic RNA in pyronin stained sections of the ventral horn cells (plate 3), shows definite changes in treated compared to control animals. While the optical density value (table 3, figure 15) relative to the cytoplasmic RNA content of anterior horn nerve cells of control animals was 0.374 ± 0.086, it was 0.398 ± 0.047 for that of 25 µg/kg , and 0.401 ± 0.060 for 50 µg/kg, 0.381 ± .046 for 75 µg/kg, and 0.367 ± 0.043 for 100 µg/kg..

In silver stained sections, the Golgi bodies appear as grayish granules in the cytoplasm of nerve cells, while the nerve fibers are stained darkly (plate 4). Measurement of the density of the golgi bodies (table 4) shows that the Golgi body content of anterior horn nerve cells of control animals was 0.2187 ± 0.036, it was 0.362 ± 0.122 for that of 25 µg /kg , and 0.351 ± 0.132 for 50 µg/kg, 0.354 ± .105 for 75 µg/kg, and 0.269 ± 0.068 for 100 µg/kg.. The general trend of the

obtained curve is similar to that of cytoplasmic RNA content (figure 16).

Changes in the myelinated fibers in the ventral horn of the spinal cord of control and treated animals have been evaluated by measurement of two factors: the change in the area occupied by these fibers relative to fixed area of the section (degenerative changes), and the density of stained myelin in these fibers (table5, figure 17). There was no change in the area occupied by the fibers . The density of silver stained myelin was inversely proportional to the dose of the drug.

Table(1): Summary Description of Arousal / Motor Coordination/ MotorTone Measures (Yaksh et al 1995)

Arousal	
+3	Continued rapid movement ; continuous squeaking ;extreme attempts to bite or escape when handled;light tactile stimulation drives vigorous squeaking ; escape / aggressive behavior ; spontaneous jumping
+2	Continuous activity ;frequent squeaking behavior ; exaggerated response to light touch (squeaking , escape, gnawing at probe
+1	Increased locomotor activity in cage ;continual grooming ;unable to position on catalepsy test
0	Normal spontaneous activity ; occasional grooming ; intermittent activity in novel environment ; rapid(<1 to 2s)recovery from catalepsy test ; orients to light touch ; no agitation displayed when picked up and strocked
-1	Decreased spontaneous activity / delayed catalepsy dismount (rat)(> 5s, < 20s) ; responds to repetitive light strocking or hand clap
-2	No spontaneous behavior ; no dismount from catalepsy bar (rat) (> 20s); delayed response to continuous pinch of fore or hind paws
-3	Comatose ; no organized response to pinch of the fore/hind paw or hand clap
Motor Coordination	
0	Normal symmetric posture , ambulates with normal symmetry ; able to spontaneously stand in grooming posture ; normal placing and stepping response; normal coordinated hindquarter righting response
-1	Ambulates asymmetrically ; weak placing and stepping response ; weak righting response
-2	Ambulates with extreme asymmetry; no placing and stepping response; one or more limbs cannot participate in righting response
-3	Unable to functionally move in a forward fashion
Motor tone	
+3	Extreme rigidity ; “barrel chest” feeling ;legs in extreme extension
+2	Moderate rigidity ; resistance to movement of the hind limbs ; increased tone observed when mild pressure is applied to the chest wall
+1	Mild rigidity ; stiffness associated with extension or flexion of limbs
0	Normal tone ; ready passive extension and flexion of hind Limb ; normal chest wall elasticity
-1	Mild decrease in tone ; limbs can be held in passive extension without resistance ; animal can retain a normal crouching posture
-2	Moderate decrease in tone ; animal in passive extension with no limb withdrawal observed after slight hyperextension ;animal unable to maintain normal crouching posture even when maximally aroused
-3	Animal has no posture tone;thoracic lordosis curvature absent; no withdrawal observed even with extreme limb extension

Table(2) : Effect of intrathecal neostigmine (2-64 µg/Kg) on the mean Arterial blood pressure of anesthetized cats (mean ± SD)

Time (min)	2 µg/kg	4 µg/kg	8 µg/kg	16 µg/kg	32 µg/kg	64 µg/kg
0	101± 2	98 ± 6	100±4	99 ± 4	100 ±3	97 ± 6
15	103 ± 4	102 3	105 ± 2*	107 ±2*	99 ± 3	95±2
30	105± 4*	107± 5*	112 ± 7**	115 ± 8**	96 ± 1	90 ± 6*
45	109 ± 2**	112 ± 6**	120 ± 4**	124 ± 2**	92 ±4**	81 ±7**
60	111 ± 5**	120 ±4**	125 ± 5**	133 ± 6**	91 ± 1**	85 ± 3**

* P<0.05 **P<0.001

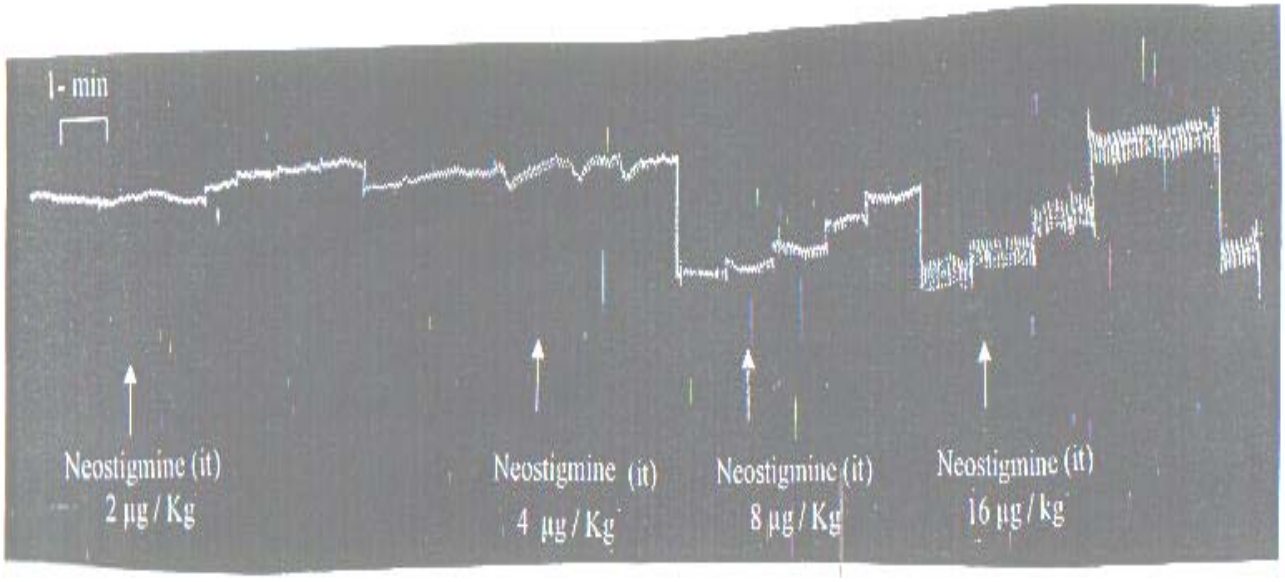

figure (1) : The effect of neostigmine injected intrathecally (it) (2- 16µg/kg) on the mean arterial blood pressure of the anesthetized cat

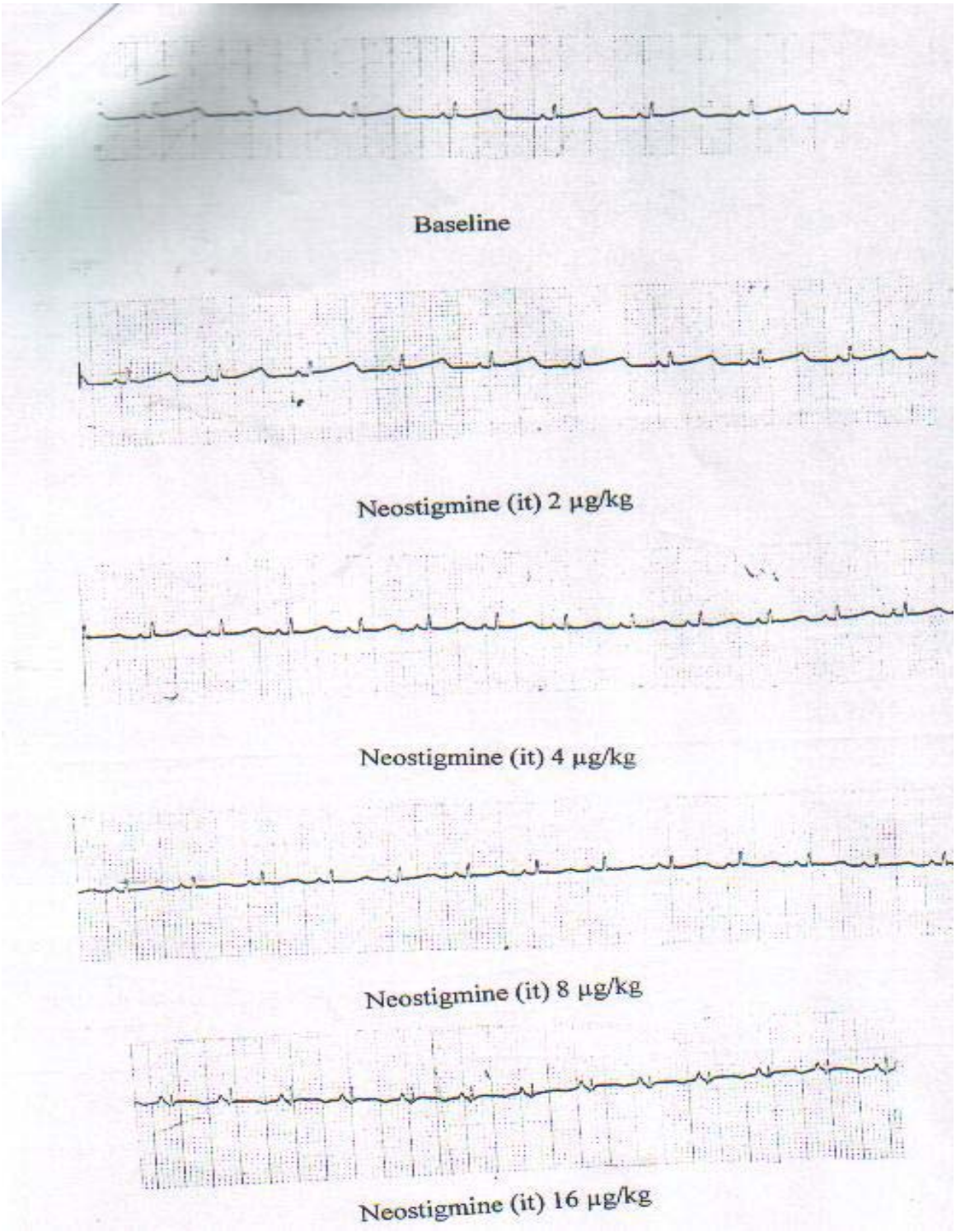

figure (2) : The effect of neostigmine injected intrathecally (it) (2- 16µg/kg) on the heart rate of the anesthetized cat.

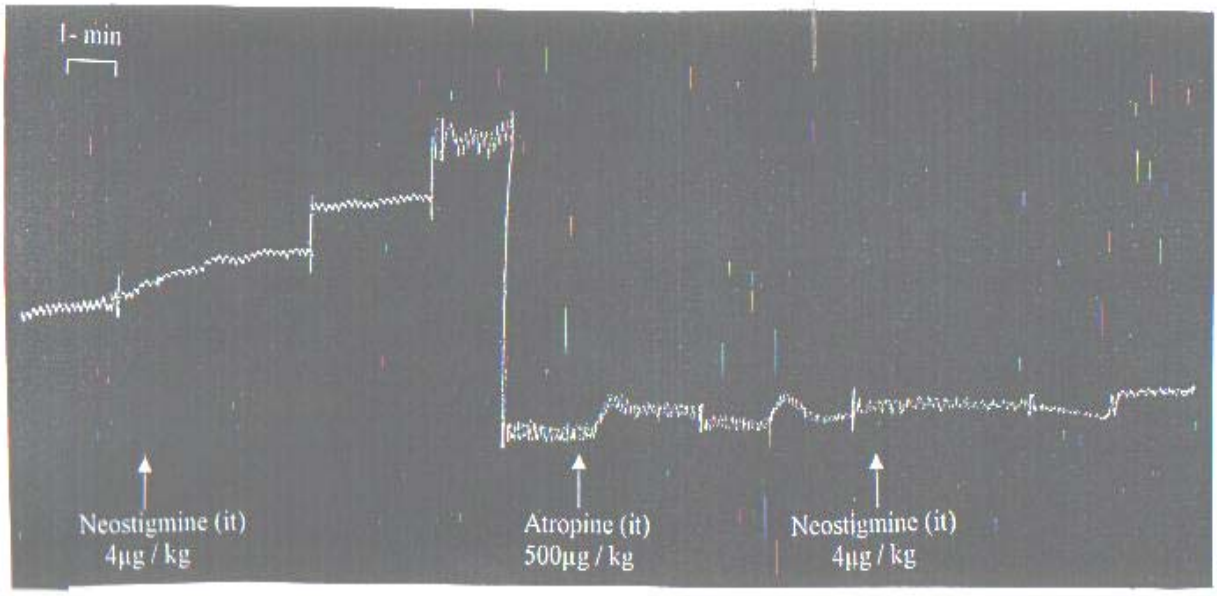

figure (3) : The effect of neostigmine injected intrathecally (it) 4µg/kg on the mean arterial blood pressure of the anesthetized cat after blocking the muscarinic receptors by atropine injected intrathecally (500 µg/kg)

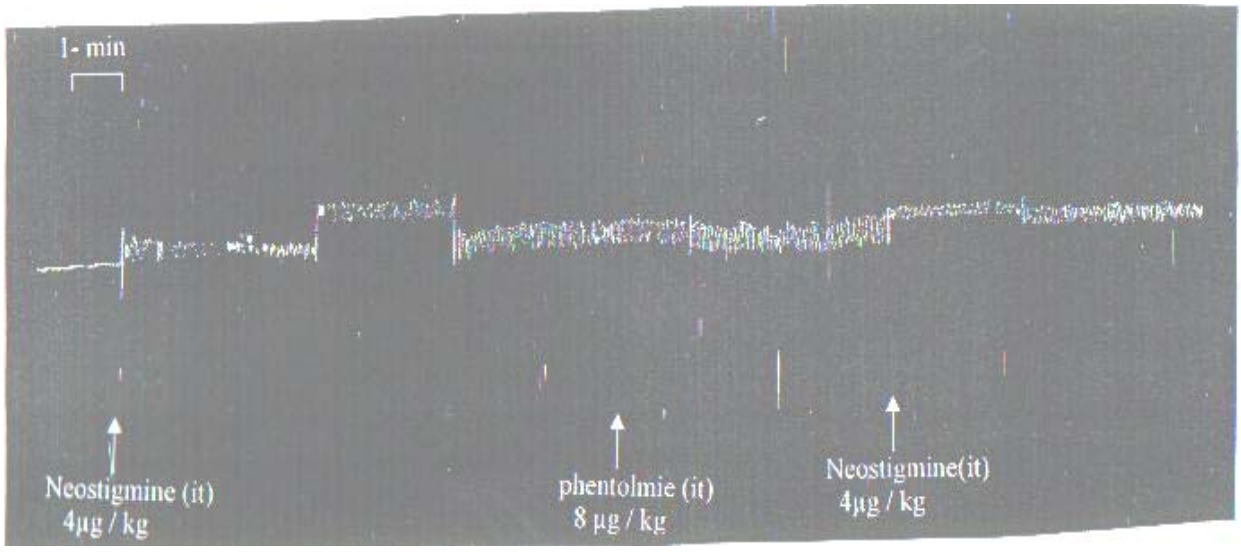

figure (4) : The effect of neostigmine injected intrathecally (it) 4µg/kg on the mean arterial blood pressure of the anesthetized cat after blocking the adrenergic receptors by phentolamine injected intrathecally (8 µg/kg)

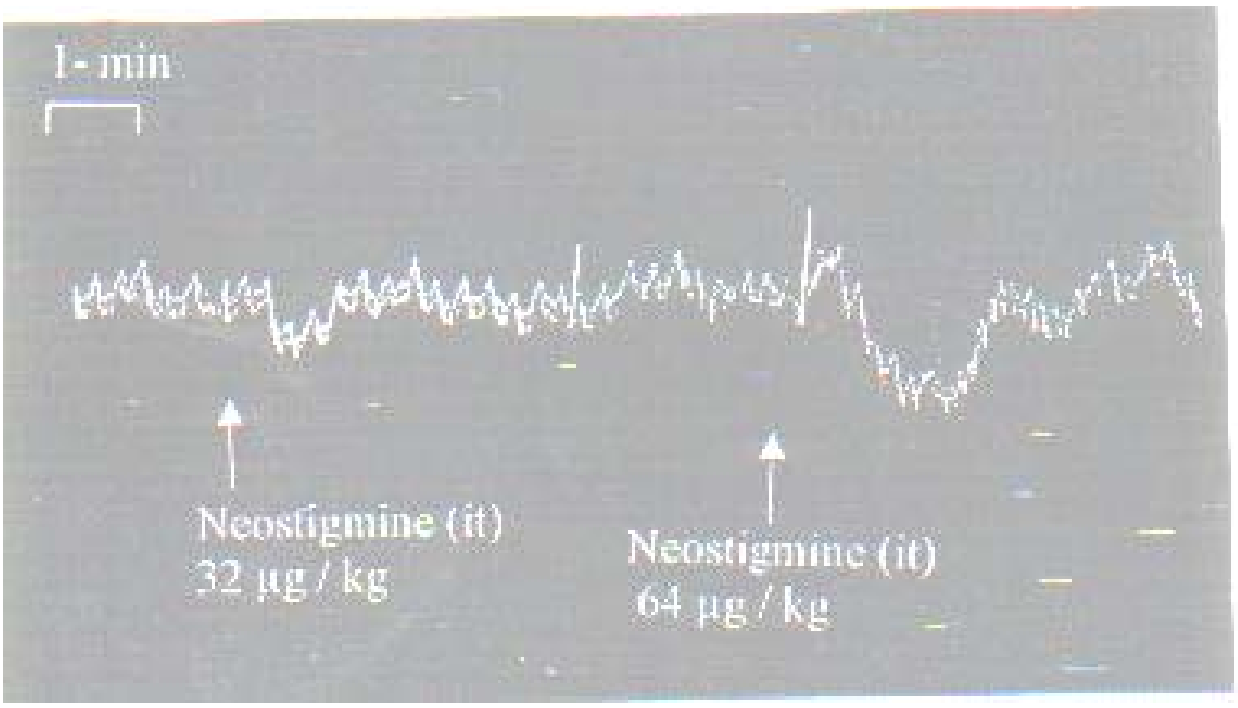

figure (5) : The effect of large doses of neostigmine injected intrathecally (it) 32µg/kg and 64µg/kg on the mean arterial blood pressure of the anesthetized cat.

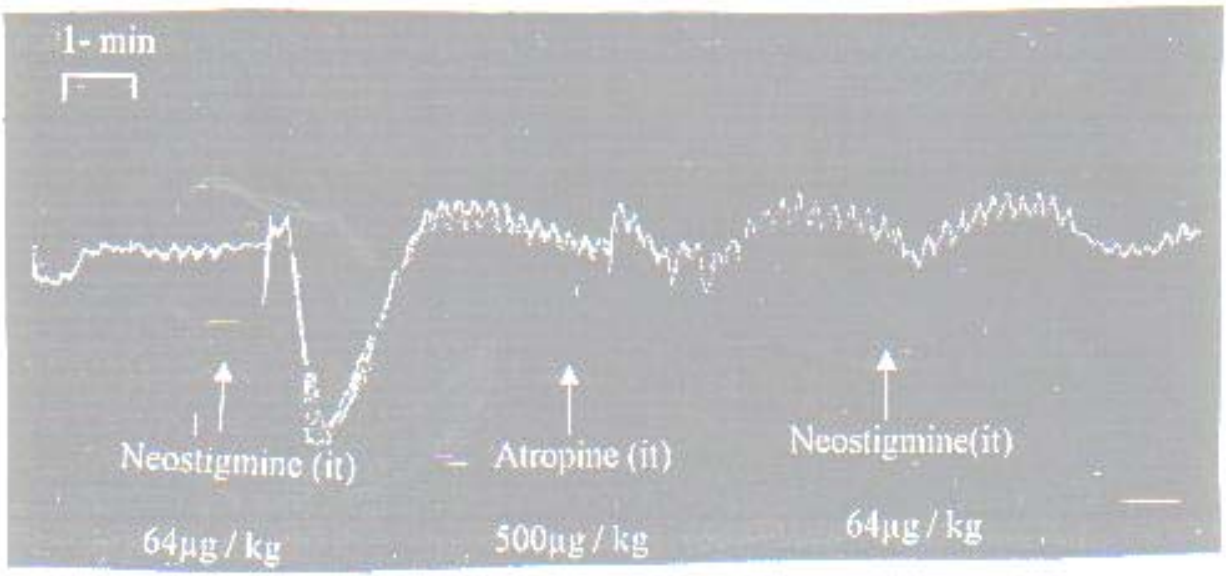

figure (6) : The effect of neostigmine injected intrathecally (it) before and after intrathecal injection of nuscarinic receptor antagonist atropine (500 µg/kg)

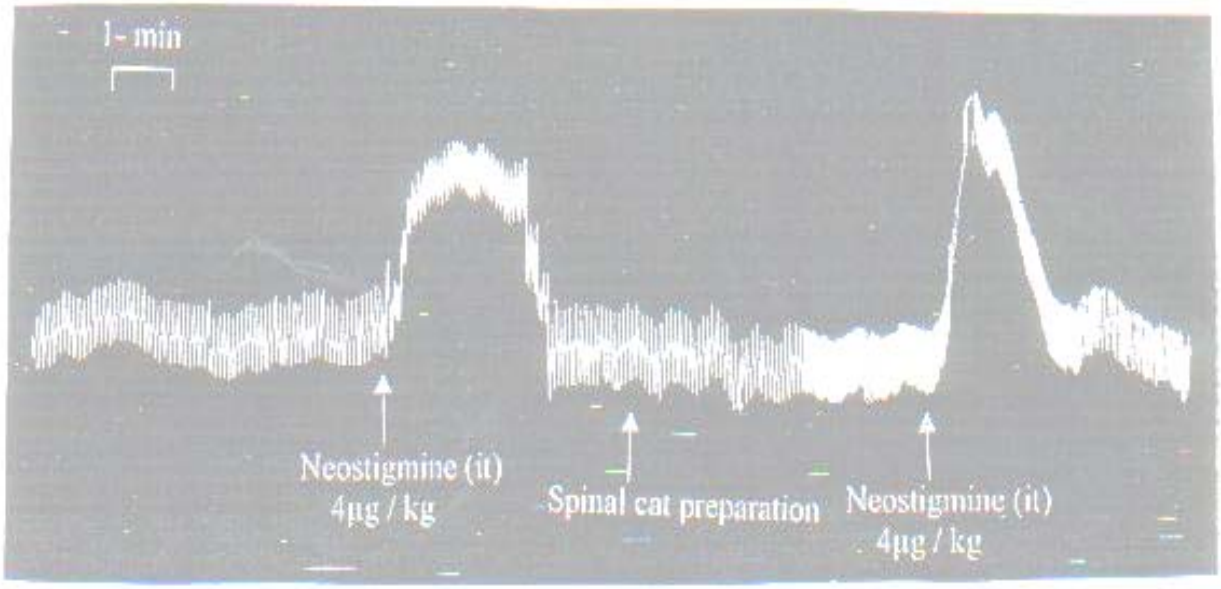

figure (7) : Spinal cat preparation, the effect of neostigmine injected intrathecally (it) (4µg/kg) before and after destruction of the brain .

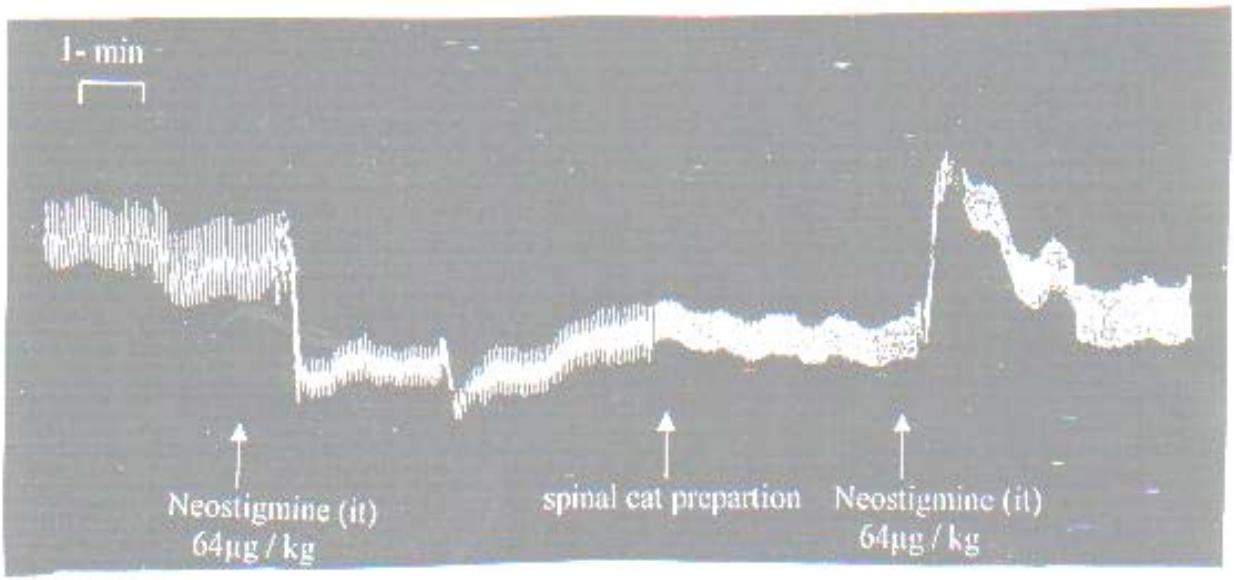

figure (8) : Spinal cat preparation, the effect of neostigmine injected intrathecally (it) (64µg/kg) before and after destruction of the brain .

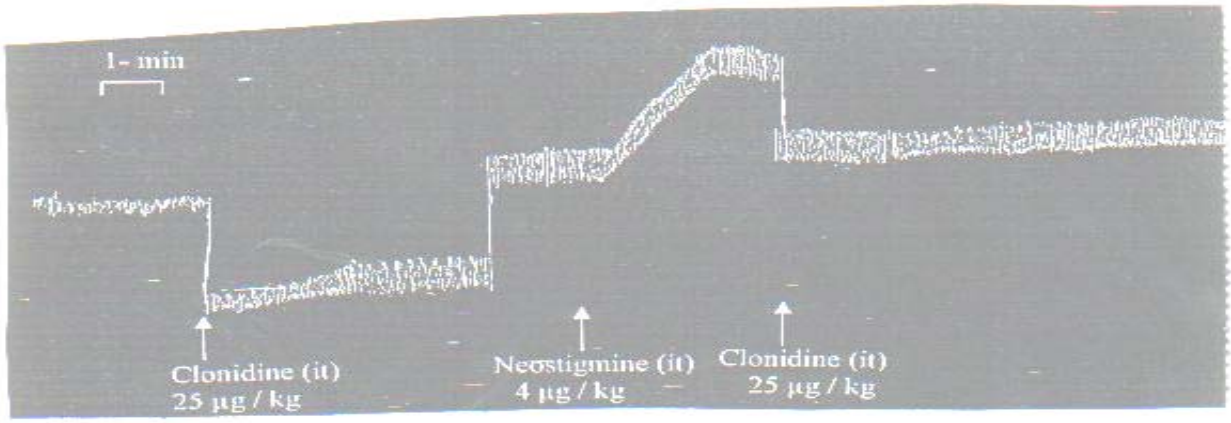

figure (9) : The interaction between clonidinde injected intrathecally (it) (25µg/kg) and neostigmine injected intrathecally (4µg/kg) on the mean arterial blood pressure of the anesthetized cat.

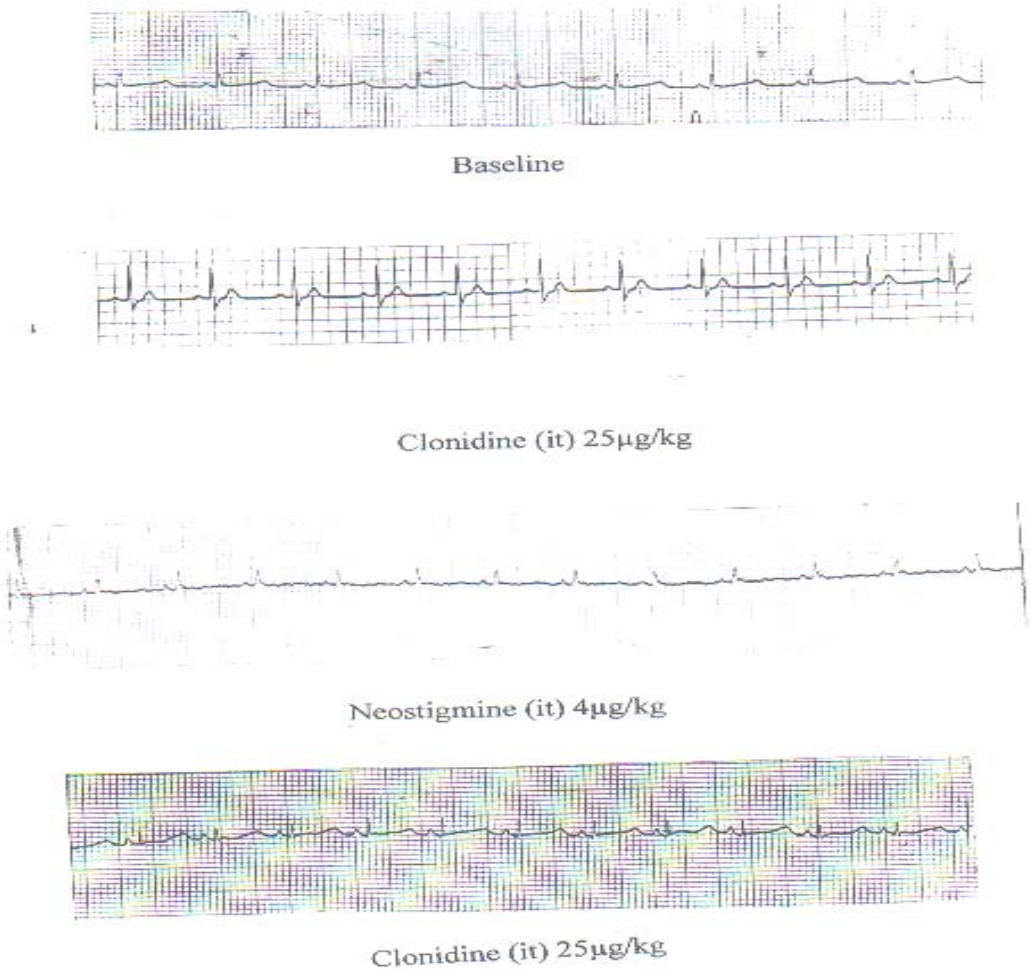

figure (10) : The interaction between clonidind injected intrathecally (it) (25µg/kg) and neostigmine injected intrathecally (it) (4µg/kg) on theheart rate of anesthetized cat.

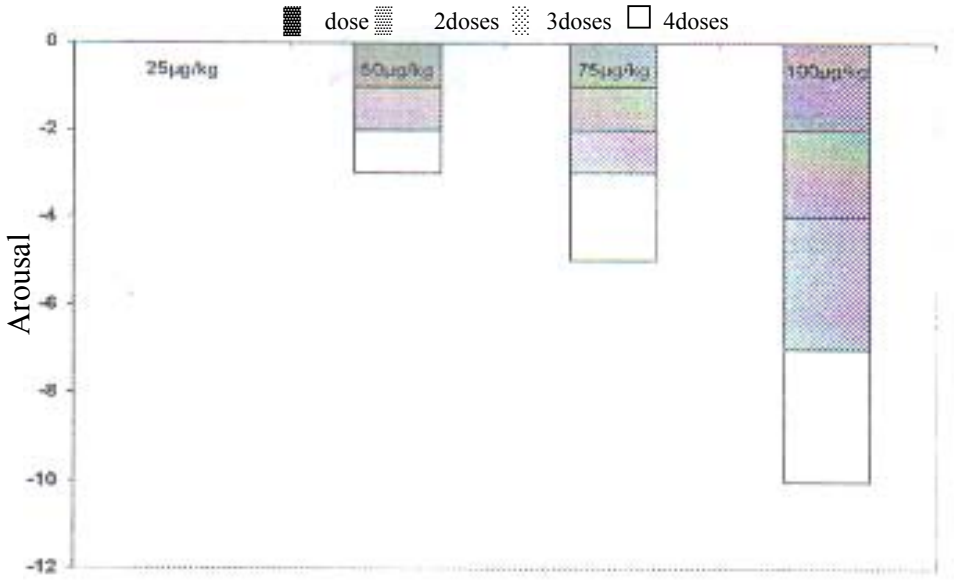

figure (11) : Effect of different doses of intrathecally injected neostigmine on the activity to no organized response to pinch of the forepaw.

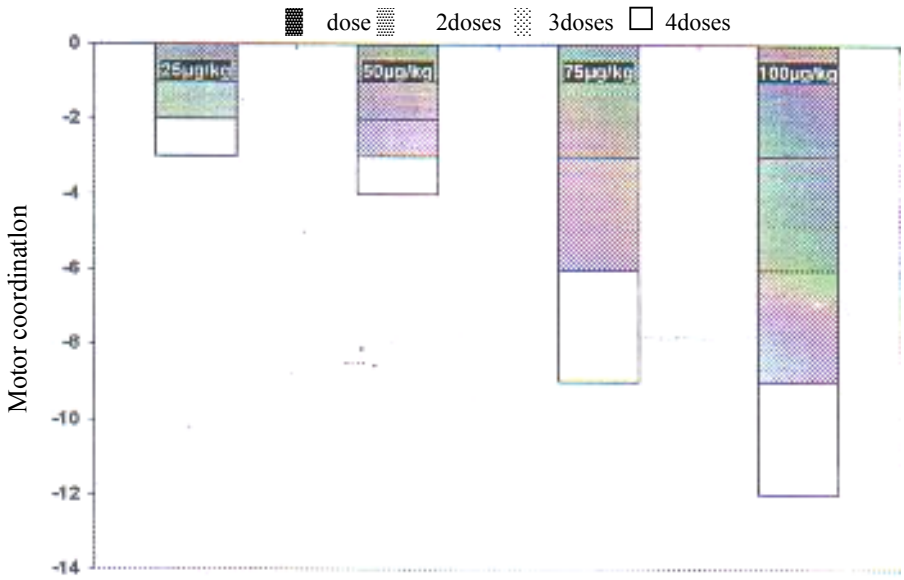

figure (12) : Effect of different doses of intrathecally injected neostigmine on motor coordination .

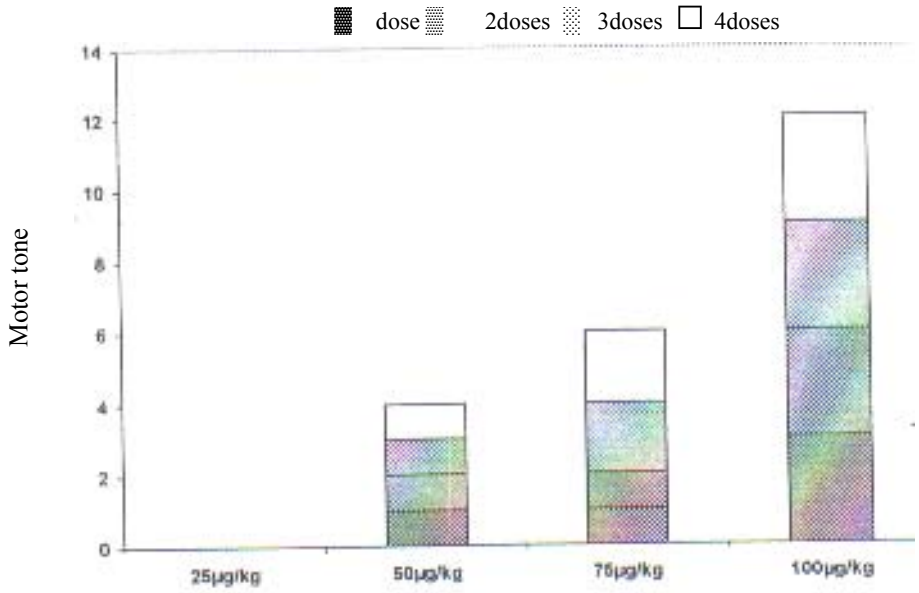

figure (13) : Effect of different doses of intrathecally injected neostigmine on motor tone .

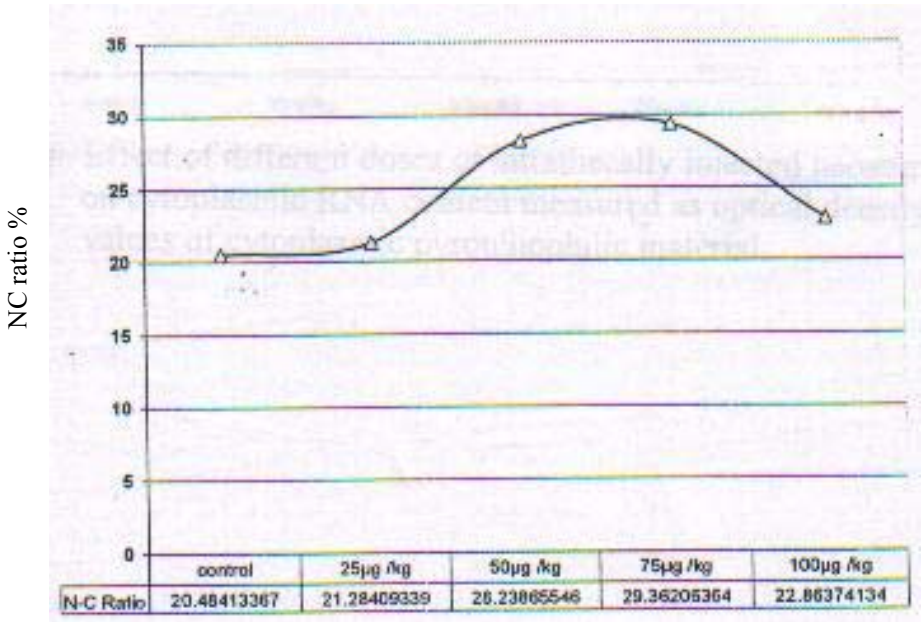

figure (14) : Effect of different doses of intrathecally injected neostigmine on nucleo-cytoplasmic ratio.

Table(3): **Mean optical density relative to pyronin stainability of Cytoplasmic RNA ± The standard deviation (SD) in control and treated animals**

	Control	25 µg /Kg	50 µg/Kg	75 µg/Kg	100 µg /Kg
Mean	0.374586	0.39829	0.401058	0.381308	0.36748
SD	0.086412	0.046789	0.060104	0.046156	0.043459
P		<0.001	<0.001	<0.001	<0.001

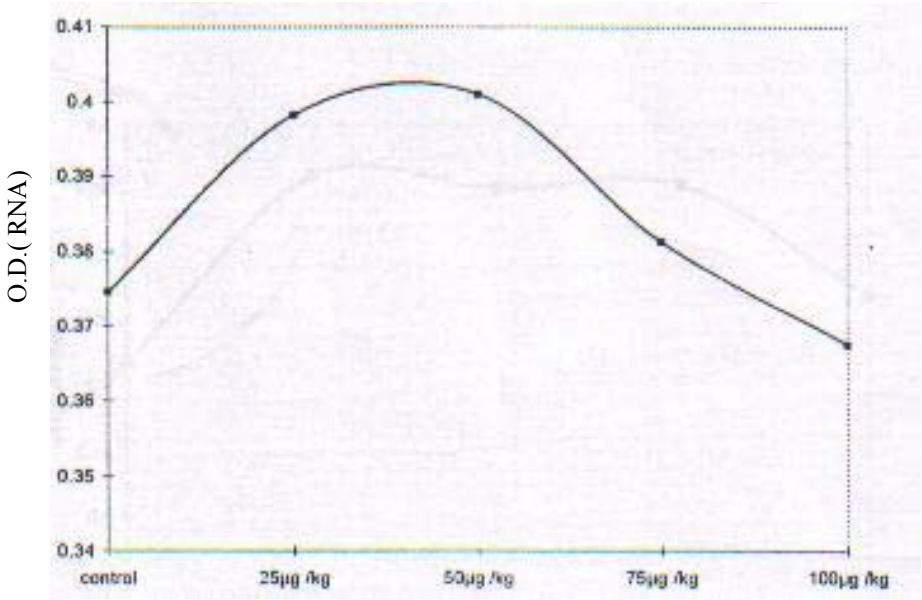

figure (15) : Effect of different doses of intrathecally injected neostigmine on cytoplasmic RNA content measured as optical density values of cytoplasmic pyroninophilic material.

Table (4) : **Mean optical density relative to silver stainability of Golgi bodies ± The standard deviation (SD) in control and treated animals**

	Control	25µg /Kg	50 µg /Kg	75µg /Kg	100 µg/Kg
Mean O.D	0.218752	0.361627	0.350981	0.354157	0.269317
SD	0.036161	0.121961	0.131755	0.105567	0.068102
P		< 0.001	< 0.001	< 0.001	< 0.001

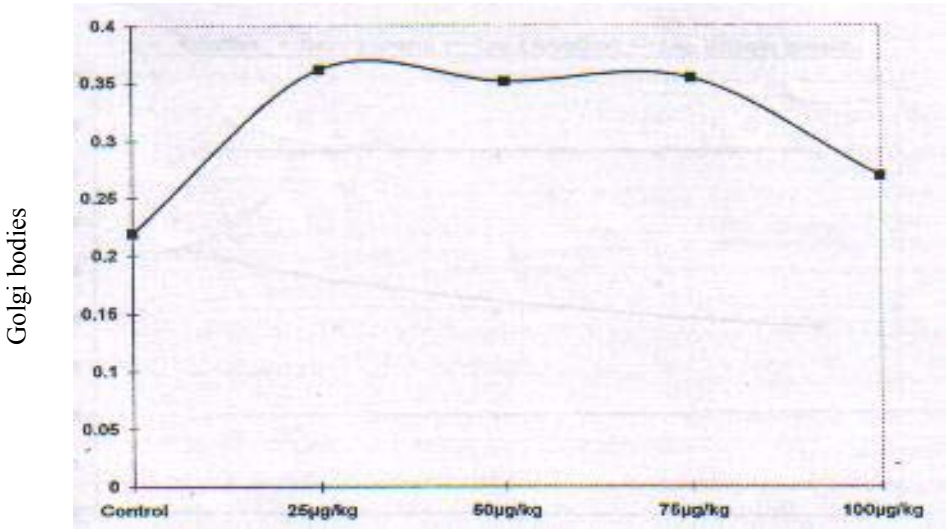

figure (16) : Effect of different doses of intrathecally injected neostigmine on Golgi bodies as measured from silver stained sections.

Table (5): **Mean area occupied by nerve fibres relative to the total area of ventral horn ± The standard deviation (SD) ;and the mean optical density relative to silver stainability of nerve fibers ± The standard deviation (SD) in control and treated animals**

	Control	25 µg /Kg	50 µg /Kg	75 µg /Kg	100µg /Kg
Area /box	0.729 ±239	0.785 ± 233	0.745± 224	0.752±228	0.744±233
Density	0.603±212	0.385±0.086*	0.418±0.076*	0.479±0.04*	0.394±0.054*

(*) The value is statistically significant compared to control .P < 0.001

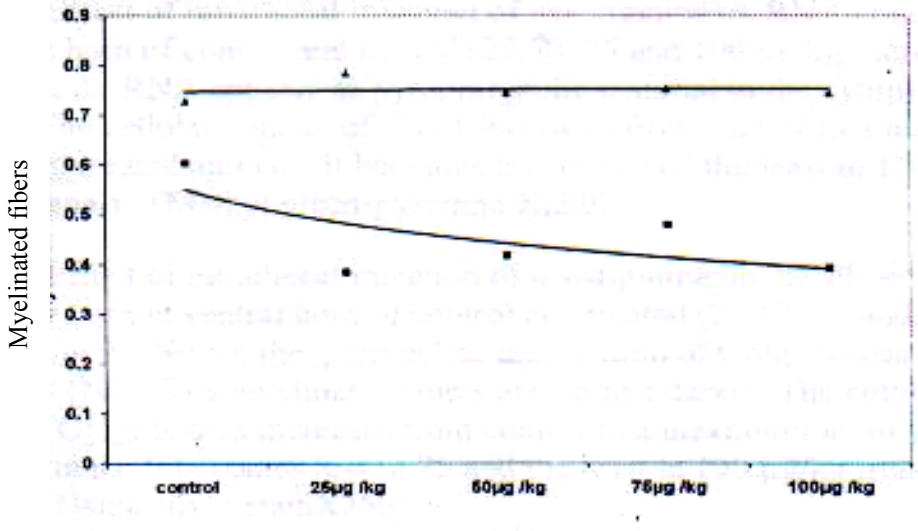

figure (17) : Effect of different doses of intrathecally injected neostigmine on the volume of myelinated fibers relative to the total tissue volume; and the density of silver stained myelin in control and treated animals.

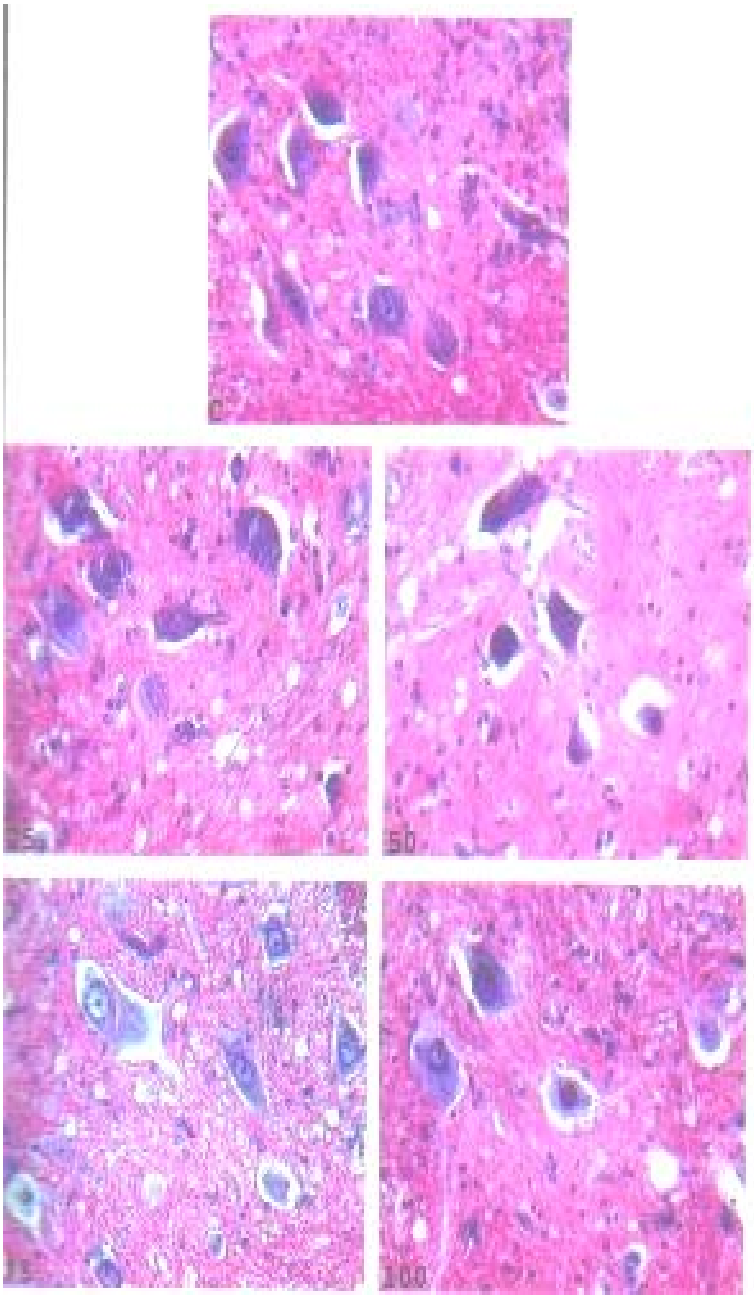

Plate (1) Effect of intrathecal injection of neostigmine on the general structurel of the ventral horn of control(C) and treated (25,50,75 and 100 μg/kg) animals .Notice the large Vesicular nuclei in the section from 75μg/kg treated animals (Hx+EX250)

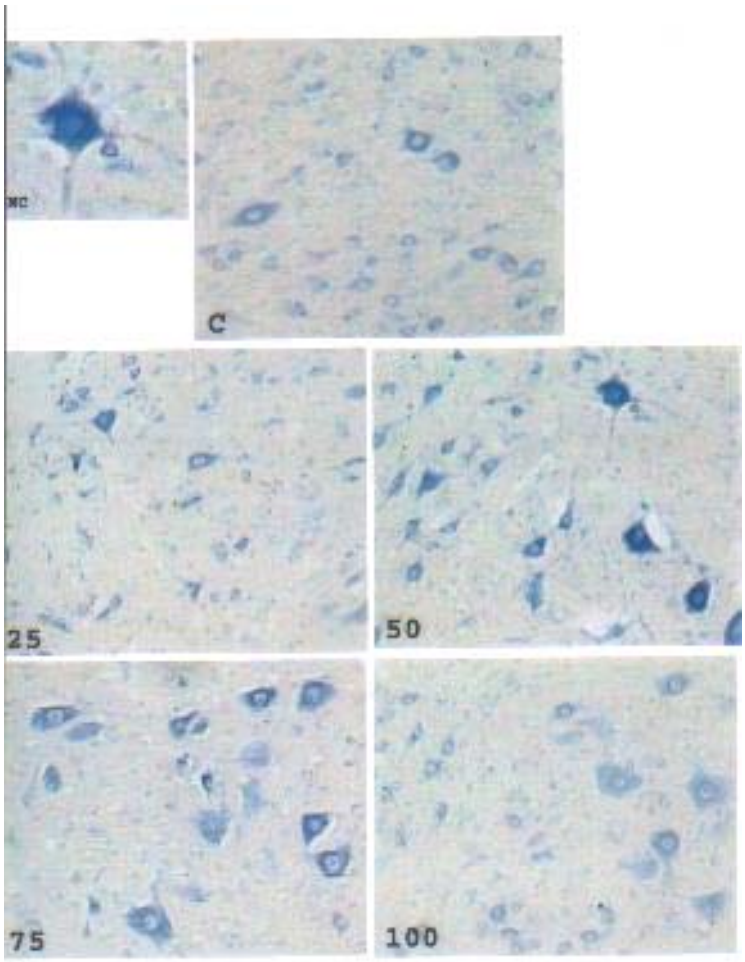

Plate (2) Effect of intrathecal injection of neostigmine on the distribution of Nissl granules of the ventral horn of control and treated (25,50,75 and 100 µg/kg) animals . Notice the perinuclear distribution of Nissl granules in nerve cells (NC) . The cellular content of Nissl granules increases from control to a maximum in 50 µg/kg treated animals .It becomes less in 75and the least in 100 µg/kg treated animals .(Toluidine blue X250)

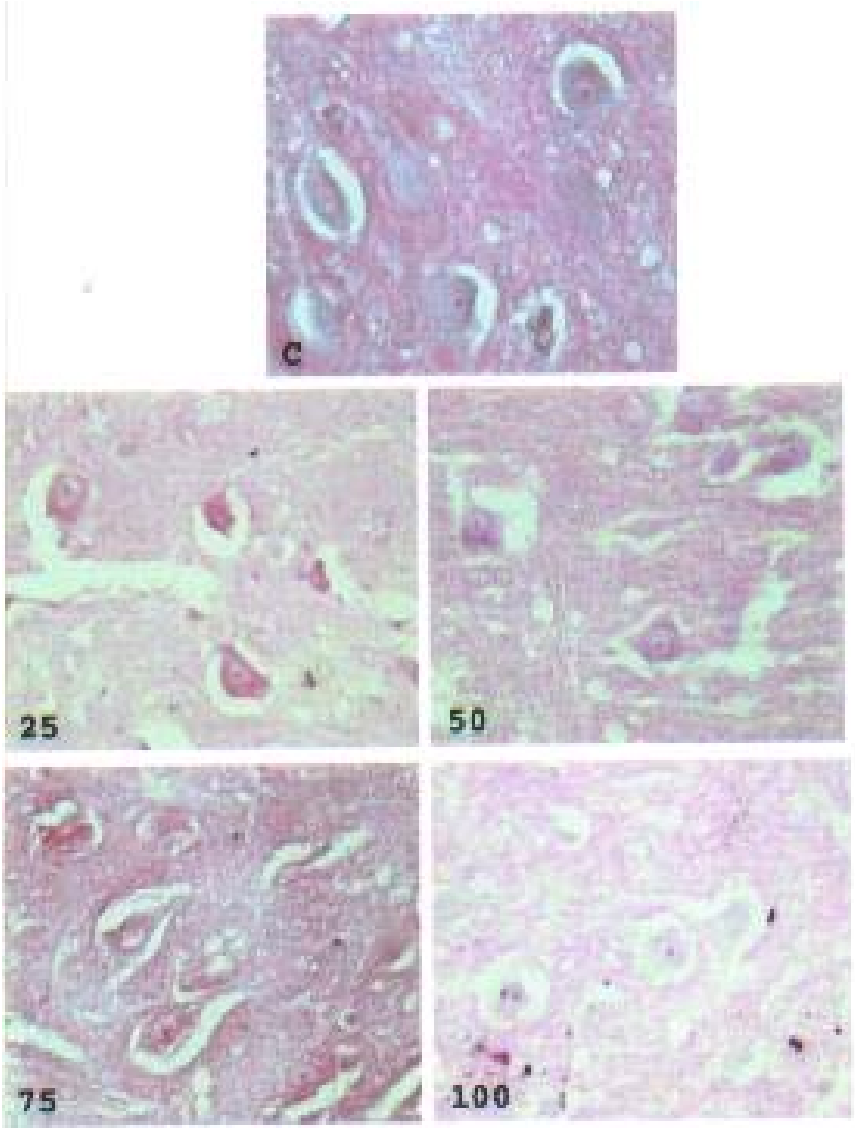

Plate (3) Effect of intrathecal injection of neostigmine on RNA content of the ventral horn of control and treated (25,50,75and100 µg/kg) animals .Notice that the RNA appears as a pyroninophilic material in the cytoplasm and nuclei .The cellular content of RNA increases from control to a maximum in 50 µg/kg treated animals .It becomes less in 75 and the least in 100 µg/kg treated animals. (Methyl green-pyronineX250) .

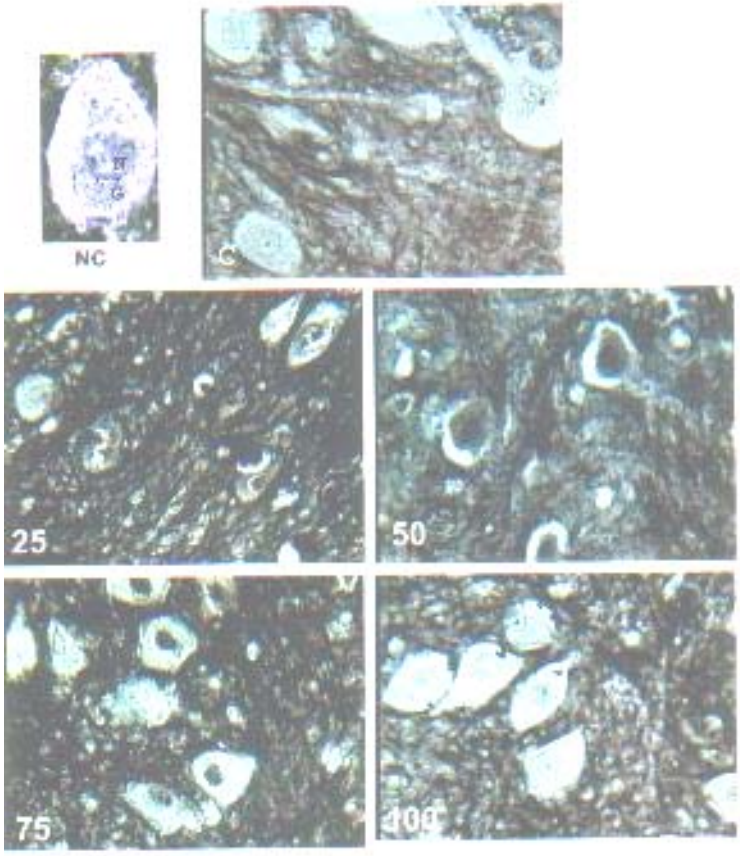

Plate (4) Effect of intrathecal injection of neostigmine on the silver stained components of control and treated (25,50,75and100 µg/kg) animals .Notice the perinuclear distribution of Golgi bodies (G) in nerve cells (NC) .The myelinated fibers are stained darkly . The cellular content of Golgi bodies increases from control to amaximum in 50µg/kg treated animals .It becomes less in 75and the least in 100 µg/kg treated animals (Nauta Silver stain X250) .

Discussion

The intrathecal injection of neostigmine into rats, resulted in dose-dependent decreased arousal and motor coordination. This effect is due the inhibition of acetyl cholinesterase activity in brain and motor end plate (13). Gillberg et al. (12) observed similar motor effects in animals receiving intrathecal neostigmine or cholinergic agonists which are thought to be due to direct actions on motor neuron out flow rather than to ischemia or neurotoxicity.

In the present study, examination of motor tone revealed that the intrathecal injection of neostigmine resulted in an

increase in motor tone which was evident by increased stiffness of the chest wall and the perception of tremors. Salivation was also noted and like motor tone this was dose dependent. These actions were considered as a reflect enhancement of spinal cholinergic activity secondary to inhibition of cholinesterase and were reported to be reversible by muscarinic antagonists (17 ,25).

The general structure of the ventral column cells and fibers of control and treated animal spinal cord shows no clear cut changes. Yaksh et al.(25) and Hood et al.(13) demons - trated that intrathecal neostigmine in large doses did not induce any histopathologic changes in the spinal cord. However, the results of the quantit -ative cytochemical and cytometric monitoring indicates signs of increased cellular activity at low doses and inhibitory action at high doses of neostigmine intrathecal injection.

the nucleo cytoplasmic ratio of the nerve cells increased as the dose increased up to 75 µg./kg of intrathecal neostigmine injection, then decreased to a level higher than control in animals injected with 100 µg / kg..

The increase plasmic ratio is a measure of nuclear activity in nucleic acid synthesis (3).. As the nerve cells are static cells that are not capable of division, and consequently DNA synth - esis (23), the nuclear activity under the influence of intrathecal injection of neostigmine must be mainly in RNA transcription. The observed changes in cytoplasmic content of Nissl granules and cytoplasmic RNA in pyronin stained sections of the ventral horn cells supports this conclusion. The obtained values of optical density of pyronin - ophilic material in the cytoplasm, which demonstrates ribosomal RNA engaged in protein synthesis, followed the same trend of nucleocytoplasmic ratio. Not only had that, but the accumulation of Golgi bodies, which is a manifestation of polypeptide processing (16), had, almost, the same trend. Collectively, the cytometric and cytochemical data indicate that intrathecal injection of neostigmine induces transcription, translation and processing of larger amounts of proteins. The protein which can be produced under these conditions is the cholinesterase enzyme. But, why should the neurons become active in cholinesterase synthesis after intrathecal injection of neostigmine?

The cholinesterase inhibitory activity of neostigmine has been reported to be due to its ability to act as a competitive inhibitor that binds to acetyl cholinesterase enzyme. By serving as alternative substrate with a similar binding orientation as acetylcholine, it gives rise to the carbamoylated enzyme. Sequestration of the enzyme in its carbamoylated form, thus, precludes the enzyme-catalyzed hydrolysis of acetyl choline for extended periods of time. The return of the acetylcholinesterase activity depends on synthesis of new enzyme (24). Actylcholinestrase enzyme was reported to be synthesized in neuron perikaryon and was localized in the cisternae of rough endoplasmic reticulum (9).

Acetylcholine is an excitatory neurotransmitter for preganglionc sympathetic neurons. Intrathecal injec - tion of cholinergic receptors agonists or cholinesterase inhibitors increases blood pressure through augmentation of sympathetic outflow (10).

In the present study intrathecal injection of neostigmine at doses 2 – 16 µg /kg induced a gradual increase in MBP of anesthetized cats which appeared 15 minutes and reached the peak 1 hour after injection. The onset latency for the effect of neostigmine could be explained by the lower lipophilicity of neostigmine resulting in longer time to its penetration of spinal cord tissue (20). Hood et al. (13) demonstrated that intrathecal injection of neostigmine produces hypertension which is mediated through local spinal actions by increasing synaptic conce - ntration of acetyl choline. Also Pan et al.(20) proposed an effect through nitric oxide production which is thought to be an important mediator of acetyl choline.

In the present study, the hypertensive effect induced by intrathecal injection of neostigmine at doses 2 – 16 µg /kg were blocked by the muscarinic antagonist atropine and the alpha antagonist phentolamine indica - ting that neostigmine injected

intrathecally acts on muscarinic receptors. These results were consistent with Caraleau et al.(5), Williams et al.(27), Feldman (10) and Pan et al. (20) who demonstrated that the cholinergic actions of neostigmine are through muscarinic receptors mainly M2 receptors in the intermediolateral cell column while the analgesic effect of the drug was through M1 muscarinic receptors suggesting that cholinergically mediated analgesia and hemodynamic effect could be separated.

In this study, neostigmine (it) at doses 32 and 64 μg /kg produced a decrease in MBP of anesthetized cats. This effect could be due to central distribution of the drug and its action at cholinergic sites in the brain or due to its systemic absorption which are related to CSF neostigmine concentration (21).

In this study, spinal cat preparation showed that the increase in MBP with small doses of neostigmine (it) could be attributed to local spinal action, while the decrease in MBP with large doses could be due to central action.

Clonidine, an imidazoline compo -und, is a selective α2 adrenoceptor agonist with an α2 : α1 activity ratio 200 : 1. Centrally acting clonidine produces analgesia by activation of descending spinal cord and supraspinal inhibitory pathways. α2 receptors in the dorsal horn of the spinal cord modulate upward transmission of nociception signals by modifying local release of the nociceptive transmitters : substance P and calcitonin gene related peptide (CGRP). The cardiovascular effects of clonidine injected intrathecally (it) involve α2 adrenoceptors and imidaz -oline receptors leading to a reduction in sympathetic tone and increase in parasympathetic tone resulting in a decrease in blood pressure and heart rate (1). Buccafusco and Margari (4) demonstrated that α2 adrenoceptor agonist and cholinergic combination is effective in producing analgesia while minimizing side effects.

In the present study, the injection of neostigmine (it) before clonidine (it) eliminates the clonidine induced hypot -ension and bradycardia. This is consistent with the previous studies of Caraleau et al. (5)who pointed out a dense blinding of cholinergic ligands within the intermediolateral wall cell column and intrathecal injection or iontophoretic application of muscarinic agonist carbacol at this site increases blood pressure and heart rate. Also Williams et al. (27) noted a dense bind -ing of α2 adrenergic ligands in the intermediolateral cell column and intrathecal injection or iontphoretic application of clonidine at this site decreases sympathetic neural activity. These authors proposed that spinal injection of neostigmine counterbal -ances clonidine effects in the interme -diolateral cell column. Conclusion:The results observed in these two species (cats&rats) demonstrated that intrath -ecal injection of neostigmine produced variations in blood pressure and heart rate of cats and alterations in cellular activity of ventral horn column of rats conclusion.

These changes were dose dependent and were attributed to the effect of neostigmine on cholinesterase enzyme activity.These results provide additional support for the clinical trials of intrathecal injection of neostigmine for analgesia .

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بعض الدراسات الفارماكولوجى و الهيستولوجي
عن تأثير حقن النيوستجمين فى داخل الأم الجافية

يؤدى حقن عقار النيوستجمين ا لمثبط للكولين استريز ا لى تسكين الألم و يزيد من تأثير العقاقير المؤثرة على حاسة ألفا أثنين أن هد ف هذا البحث هو أجراء بعض الدراسات الفارماكولوجى و الهيستولوجى لتقييم هذا العقار فى القطط والفئران .وقد أظهرت النتائج أن حقن النيوستجمين داخل الأم الجافية للقطط قد أدى الى زيادة تدريجية فى متوسط ضغط الدم و معدل ضربات القلب عند الجرعة من اثنين الى ستة عشر ميكروجرام لكل كيلوجرام بينما تناقص متوسط ضغط الدم و معدل ضربات القلب عند الجرعات من اثنين و ثلاثين الى اربعة و ستين ميكروجرام. وقد ادى حقن الأتروبين والفينتولامين الى الغاء زيادة متوسط ضغط الدم و معدل ضربات القلب نتيجة حقن عقار النيوستجمين داخل الأم الجافية (أربعة ميكروجرام لكل كيلوجرام) واوضحت هذه الدراسة ان حقن عقار النيوستجمين بعد تدمير الحبل الشوكى للقطط قد أدى الى تزايد سريع بمتوسط ضغط الدم عند الجرعة الصغيرة (اربعة ميكروجرام لكل كيلوجرام) والجرعة الكبيرة (اربعة وستين ميكروجرام لكل كيلوجرام) . ذلك اظهرت نتائج هذا البحث ان حقن غقار النيوستجمين داخل الأم الجافية قد أبطل مفعول عقار الكلونيد ين على الضغط و معدل ضربات القلب بااقطط و قد أجريت الدراسة الهيستولوجى على الفئران وتم تقسيمها الى خمسة مجموعات :مجموعة حاكمة للمقارنة بها و اربعة مجموعات تم حقنها بعقار النيوستجمين داخل الأم الجافية عند جرعات خمس وعشرين،خمسين، خمس و سبعين ومائة ميكروجرام لكل كيلوجرام .عند تقييم سلوك الفئران بعد كل جرعة وجد انه قد أدى حقن عقار النيوستجمين داخل الأم الجافية فى الفئران الى نقصان يقظة وتناسق حركة عضلات الفئران بينما زادت قوة شد هذه العضلات.

وتبين ان هذا التاثير يعتمد على جرعة النيوستجمين السابق حقنها. واظهرت النتائج تغيرات فى الخلايا العصبية للقرن الأمامى للحبل الشوكى فى الفئران بينما لم يغير من مقدار الألياف العصبية بالحبل الشوكى بينما انخفضت حدة تشرب هذه الألياف بصبغة الفضة وكان لجرعة النيوستجمين أثرها على هذه النتائج .

ونستخلص من هذا البحث أن مضاعفات حقن عقار النيوستجمين داخل الأم الجافية يعتمد على الجرعة السابق حقنها و التى لابد أن يكون لها أهميتها فى التطبيق العملى