ACUTE EXPOSURE OF PERIPHERAL NERVE TO NEOMYCIN AND NERVE CONDUCTION

by

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SUMMARY

Since the isolation of neomycin from Streptomyces fradiae by Waksman and Lechevalier in 1949, neomycin has been successfully in treating infection caused by gram-negative bacteria. Beside the therapeutic significance, the aminoglycoside is also well known of its ototoxicity, nephrotoxicity, neuromuscular blockade effect and modulation of the normal function of several excitable tissues (Schtbeci and Schacht, 1977; Schacht, 1986; Hawkins, 1976 and Baumann, Hamann and Leung,1988). Recently, streptomycin has been used successfully in the treatment of idiopathic trigeminal neuralgia (Sokolovic, Todorovic, Stajeic and Petrovic 1986). From the works of the above authors, the aminoglycoside may act diversely on cellular mechanisms. The aim of the present study is to investigate the possible effects of neomycin on the conduction in nerve fibres.

Frog sciatic nerve and rat ventrolateral tail nerve were used to investigate the effect of acute exposure of neomycin on nervous conduction. In vitro study of the A compound action potential was performed in frog sciatic nerve. Amplitudes as well as the conduction velocities were measured. There were no statistically significant difference in change of amplitudes and conduction velocities before and after

the application of neomycin solution. In the present experimental set-up, blocking effect of the A potential of the frog sciatic nerve was shown with the application of 20 mM KCL in isotonic ringer solution. In vivo study of A & C compound action potential were carried out in the rat ventrolateral tail nerve. In the A compound action potential, there were no statistically significant difference in change of amplitudes and conduction velocities before and after the application of neomycin in Krebs solution. In the C compound action potential, there were no statistically significant difference in changes of amplitudes and conduction velocities before and after the application of neomycin in Krebs solution.

In the present experimental arrangement, 10 mM K_2SO_4 in Krebs solution shows a complete blockade of the A potential in 5-10 minutes. In the unmyelineated nerve fibre, the blocking effect of the C potential is slower and smaller than the myelinated nerve fibre.

The results from the present study indicate that with the present experiment arrangement, neomycin does not produce any acute functional impairment of nerve conduction in the myelinated and unmyelinated nerve fibres, even in the present of a fairly high concentration (3500 mg/l). It is concluded that the

action of neomycin on the nerve fibre behave differently to that on the neuromuscular junction, lateral line organ and Merkel cell receptor.

INTRODUCTION:-

Neomycin belongs to the aminoglycoside group of antibiotics. It is distinct from others by having three sugar rings rather than 2, one of them being the pentose ribose (ring III). It is produced by Streptomyces fradiae and was first isolated in 1949 (Waksman and Lechevalier, 1949). The group is used primarily to treat infection caused by gram negative bacteria. Aminoglycosides can act to interfere with protein synthesis in susceptible microorganisms (Pestka, 1971; Sande and Mandell, 1985), changes in cell permeability and transport (Hancock, 1981) or misreading of the genetic code (Pestka, 1971). Besides these therapeutic effect, the toxicity of the aminoglycosides include neuromuscular blockade (Adams and Matthew, 1973; Singh, Harvey and Marshall, 1978; Rosayro and Healy, 1978; Fickers,1983), nephrotoxicity (Schtbeci and Schacht, 1977), ototoxicity (Hawkins, 1976) as well as reduction of responsiveness in touch corpuscles (Baumann, Hamann and Leung, 1986). Recently, streptomycin had been shown successfully in the treatment of idiopathic neuralgia (Sokolovic, Todorovic, Stajeic and Petrovic, 1986).

The polyphosphoinositides were first isolated from brain by Folch in 1949. They are synthesized by sequential kinase reaction from phosphatidylinositol and catabolized either to phosphatidylinositol by

monoesteric cleavage of the phosphate groups or to diglyceride and the respective inositol phosphates by a phosphodiesterase. It plays an important role in signal transduction from the receptors at the plasma membrane. The link between inositol lipids and calcium signalling was first recognized by Michell who noticed that all agonists that seemed to use calcium as an internal signal were also capable of stimulating the hydrolysis of inositol lipids (Michell, 1975).

It has been suggested that the lack of inositol and resulting metabolic disturbances are responsible for the nerve conduction defects in diabetes (Winegrad 1983). The concentration of free inositol is reduced in sciatic nerve of streptozotocin-diabetic rats (Greene, De Jesus and Winegrad, 1975; Palmano, Whiting and Hawthorne, 1977) and in post-mortem nerve samples from diabetic patients (Mayhem, Gillon and Hawthorne, 1983). Whether these changes account for the nerve conduction defect of diabetes need further studies.

There is clear evidence of calcium fluxes as well as the well known sodium and potassium fluxes associated with the action potential in nerve potential. Is the polyphosphoinositides metabolism involved in the permeability changes accompanying nerve impulse? Little is known about this possible mechanism.

Neomycin has been used as a tool to study the polyphosphoinositide metabolism (Schacht, Weiner & Lodhi 1978). It has been shown to inhibit phosphoinositide turnover in a number of tissues by directly binding to phosphatidylinositol 4,5 bisphosphate (PIP2) and phosphatidylinositol 4-phosphate (PIP) (Schacht 1976, Lodhi, Weiner and Schacht, 1979). At low concentration, neomycin selectively binds to PIP2 over PIP (Lodhi et al., 1979).

Despite the modulating effect of the aminoglycoside on many of the excitable tissues, little knowledge is available in its action on the nervous conduction in peripheral nerve. Neomycin is employed in the present study to investigate whether acute exposure to neomycin would affect the nerve conduction in peripheral nerve.

SECTION I:- LITERATURE REVIEW

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1.1 Structure of peripheral myelinated nerve fibres

Peripheral myelinated axons are elongated cell processes derived from neuronal cell bodies in the spinal cord or brain stem motor nuclei, in the spiral and cranial sensory ganglia and in the autonomic ganglia. The myelinated axons are surrounded by the tubular myelin sheath which extends from a point near the cell body excluding the initial segments and ends at about 1 to 2 um from the axon terminals. The myelin sheath consists of a series of cylindrical segments each being termed an internode and derived from an individual Schwann cell. The junction between successive Schwann cells are called the nodes of Ranvier.

The Axon

Axolemma

The axon is bounded by a surface membrane, the axolemma. On electronmicroscopic studies, it is an asymmetric trilaminar membrane 7 to 8 nm in thickness. The inner leaflet is more osmiophilic and slightly thicker than the outer and the leaflets are separated by a clear zone (Elfvin, 1961; 1963).

Axoplasm

The axoplasm is the fluid cytoplasmic material inside the axon. Inside there is a variety of filamentous structures and organelles. Fixed silver-stained preparations of peripheral axons viewed by light microscopy show longitudinally oriented fibrillar material (Beer, Schmitt and Young, 1937). Electron microscopy subsequently established the presence of microtubules (neurotubules) and neurofilaments. The microtubules are elements of the cytoskeleton of the axon and probably involved in axonal transport mechanisms. This structure is cyclindrical in appearance with a diameter of average 25 nm and a wall of 5 nm in thickness surrounding a hollow core, sometimes containing 5 nm granules. They are occasionally interconnected to neighbouring organelles such as mitochrondia or neurofilaments. The axonal microtubules are more marked in the region where the neurofilament density is reduced and may be associated with mitochondria and cisternal of smooth endoplasmic reticulum (Raine, Ghetti and Shelanski, 1971). The neurofilaments are unbranched longitudinally oriented structures with an average diameter of 10 nm and uncertain length (Wuerker and Kirk, 1972). Their function is not understood adequately. They may be involved in axonal transport mechanism.

The mitochondria originate from the cell body and are then slowly translocated down the axon. Time-lapse

photography of living amphibian axons shows rod-like particles that are probably mitochondria, which exhibit jerky movements in either direction or move slowly in an antero-grade direction at a rate of 1.5 mm/day (Cooper and Smith 1974). The mitochondria are more numerous in small axons (ranging from 2 to 5 /um) than in larger axons (average 0.1 um).

The axonal smooth endoplasmic reticulum is seen as discrete tubular or flattened cisternae in transverse or longitudinal section. It is a continuous reticular meshwork extending from the axon hillock to the nerve terminals and are occasionally expanded in the form of flattened subaxolemmal plates. (Droz, Rambourg and Koenig, 1975).

The periaxonal space is a space between external aspect of the axolemma and the plasmalemma of adaxonal Schwann cell. It occupies a space of about 15 to 20 nm. It may be arranged in 5 or 7 layered configuration with a 2 to 4 nm intramembranous space.

Schwann cell

The Schwann cell can be considered as a flattened, trapezoid-shaped cell that is wrapped spirally around the axon. It can be divided into three portions. The adaxonal cytoplasmic margin constitutes the innermost portion while the ab-axonal cytoplasm or " outer belt" that surrounds the myelin sheath and bears the nodal

microvilli at its margin in the outer portion. The intermediate part constitutes the compact myelin, the lateral cytoplasmic rim of which has a helical attachment to the axolemma at the paranode. The myelin is traversed by cytoplasmic channels, the incisures of Schmidt-Lantermann, most of which interconnect the adaxonal and abaxonal cytoplasm. The adaxonal Schwamm cell cytoplasm runs in a spiral manner that executes slightly more than one complete turn. At the region of overlap, the apposition of the adjacent surface membranes is termed the mesaxon. The amount of abaxonal cytoplasm is frequently limited, the surface membrane of the Schwann cell closely overlying the outermost myelin lamella. It again consists of a spiral process.

The myelin sheath

The myelin sheath is a membrane system which is characterized by highly regular construction composed of spirally arranged lamellae. Each individual lamella arises by the spiral wrapping of Schwann cell plasmalemma around that axon during myelination. From the polarization optical findings on the myelin sheath and with the results of X-ray diffraction studies (Schmitt and Clark, 1935), the myelin sheath consists of alternating cylindrical layers of lipid and protein. In this arrangement, the lipid forms a bimolecular leaflet oriented with the hydrophobic ends directed toward the

center and with the hydrophilic polar ends extended outward and sandwiched between monolayers of protein. This concept of the myelin sheath configuration was supported by ontogenetic studies of myelin in chick embryo (Geren, 1954) and high resolution electron microscopic analyses (Robertson, 1955).

The Node of Ranvier

In 1871, Ranvier observed that the myelin sheath was indented at regular intervals. The essential feature of the nodes was a complete interruption of the myelin. The terminal part of the myelin approaches the nodal axon at an acute angle in the smaller fibres while on the larger fibers, the ends of the myelin segments form bulbous expansions and the terminal myelin approaches the axon more steeply, sometimes being recurved so that it forms an angle in excess of 90°.

The part of the axon which is exposed at the node of Ranvier i.e. the nodal axon, is narrowed. The relative degree being greater for the larger fibers.

The paranodal region

The terminal cytoplasmic rim of the myelin lamellae is firmly adherent to the axolemma at either side of the node. The axolemma at the junction with the helical rim

is specialized. This portion of the axon is designated the paranodal region and separates the nodal and internodal portions of the axon. Fracture faces through the paranodal region disclose a helical ridge and trough pattern conforming to the apposition of the Schwann cell terminal cytoplasmic helix (Schnapp and Mugnaini,1978).

The paranodal apparatus may be involved in impulse generation. It seems that the area of the exposed nodal axolemma is insufficient to accommodate the number of sodium channels found from saxitoxin binding experiments (Wiley and Ellisman, 1980) and it was proposed that the exchanges of sodium at the nodes utilize a "synaptic" junction in the paranodal region. Ellisman and co-workers found an elevated concentration of sodium in the helical cytoplasmic rim of Schwann cell in the paranode (Ellisman, Freedman and Hamilton, 1980). They postulated that this portion of the Schwann cell provides the source for the inward flux of sodium ions that occurs through the nodal axolemma during generation of the action potential. The sodium is then pumped out by the nodal axolemma and taken up from the nodal gap by the Schwamm cell nodal microvilli from where it moves to the paranodal cytoplasmic helix.

Unmyelinated nerve fibre

In mammals, there are approximately 75% unmyelinated axons in cutaneous nerves and dorsal spinal

roots (Ochoa and Mair, 1969), 50% in muscle nerves and 30% in ventral roots (Coggeshall, Coulter and Willis, 1973; 1974). White rami communicantes contain approximately two-third unmyelinated axons (Coggeshall, Hancock and Applebaum, 1976).

The axoplasm

The axoplasm of unmyelinated axons is qualitatively similar to that of myelinated axons and also to that of immature axons. The concentration of neurofilaments is comparable but that for microtubules is greater than the myelinated axons (Berthold, 1978). Elongated mitochondria and dense cored vesicles occur but not abundant in unmyelinated axons (Ochoa, 1976).

The schwann cell

The satellite cells associated with unmyelinated axons and those associated with myelinated axons have a common embryological origin. Their structural features show many similarities. The nuclei of satellite cells associated with unmyelinated axons are elongated and smooth in contour, aligned longitudinally like a series of bacilli and possess one or more nucleoli. The cytoplasm of the satellite cells contain a variety of organelles in the perinuclear region while elsewhere in the cytoplasm only occasional filaments, microtubules and sparse mitochrondia. Elements of the golgi complex

are consistently found near the nucleus of Schwann cells. (Ochoa, 1976).

The whole surface of unmyelinated axons in normal mature somatic nerves is invested by Schwann cell process. When a Schwann cell process fails to cover part of the surface of a peripheral axon, there is still a layer of basal lamina separating the axon from the endoneurium. Short isolated Schwann cell processes located externally within the axonal-Schwann cell complexes are the "collegan pockets". This term was described by Gamble and Eames (1964) in peripheral nerves of rats and human and are exclusive features of unmyelinated fibres (Gamble, 1964)

1.2 Relationship between myelin thickness and axon diameter

Studies on the consequences of cross anastomosis between myelinated and unmyelinated nerves indicate that it is the axon that provides the stimulus for the Schwann cell to produce myelin (Hillarp and Olivecroma, 1946). There are controversial reports related to myelin thickness against axon diameter. Sanders (1948) found that there is a linear relationship between myelin thickness and axon diameter . Other workers have found either a direct rectilinear correlation between myelin thickness and axon diameter (Williams and Wendell-Smith,

1971) or a relatively greater myelin thickness for fibres of smaller diameter (Evans and Vizoso, 1951). on the other hand, Buchthal and Rosenfalck (1966) found that myelin thickness was proportionally greater in larger fibres. The ratio (g) between the total fiber diameter and axon diameter derived by Schmitt and Bear in 1937 had been found useful in theoretical treatment relating to saltatory conduction. Rushton (1951) predicted that a value of 0.6 to 0.7 for g would be optimal for conduction velocity and was subsequently reiterated by Hodgkin in 1967 and Smith and Koles in a computer simulation in 1970).

1.3 Classification of nerve fibres in peripheral nerve

The existing classification of nerve fibres in peripheral nerves are based either on the conduction velocities or on fibre diameters. In the studies of electrical stimulation of frog sciatic nerve, Erlanger and Gasser (1937) used A plus Greek letters alpha, beta, gamma, delta to identify successive peaks of the action potential in order of decreasing conduction velocities. Subsequently, two additional peaks produced by fibres of much lower conduction velocity were discovered and were called B and C fibres. The A potential are produced by myelinated fibres ranging from large to small diameters, In mammalian nerves the B wave are considered to be action potential of the preganglionic (small diameter myelinated) fibers in autonomic nerves. In peripheral

nerve, the A delta is applied to the B conduction velocity in the sympathetic fibers. The C-potential is produced by the unmyelinated fibers (Erlanger and Gasser, 1937; Keynes, 1972).

An alternative classification for afferent nerve fibres was based on the fibre diameter (Llyod, 1943). Based on the distribution of fibre diameters, there are 4 different fibre groups (range from Group I to IV) in somatic nerves. The fibres diameter and their corresponding level with the Erlanger and Gasser classification is as follow:

Cutaneous nerve	muscle nerve	conduction vel. in cat	Diameter
A alpha & beta	Gp.I	72-130 m/s	12-22μm
		35-108 m/s	6-18μm
	Gp.II	36-72 m/s	6-12μm
A delta	Gp.III	3-30 m/s	3-7μm
C	Gp.IV	0.2-2 m/s	0.25-1.3um

(From Light and Perl, (1984) in Peripheral Neuropathy pp. 213).

These two classifications have lead to confusion and controversy on how to use them. Some workers use Llyods (1943) classification to all afferent

fibres and Erlanger and Gasser's (1937) classification for the efferent fibres (Jaenig and Zimmermann, 1971; Bowsher, 1970). Thus afferent fibres from muscles were subdivided into its Group I, II, & III by Llyod's system. Cutaneous afferent myelinated fibres fall into 2 groups namely II and III since the mean diameter of the large cutaneous afferents is considerably less than that of Group I muscles afferent. These 2 groups of fibres correspond to the A alpha and A gamma as dervied by Gasser's classification. However, the initial peak called A alpha by Gasser was called A beta by Erlanger because its conduction velocity was like that of the A beta peak in muscle nerve. The unmyelinated fibres produce with later peak called C and was correspond to Group IV fibre by the Llyod classification.

1.4 Relationship between fiber diameter and conduction velocity

Gasser and Grundfest (1939) studied the compound action potentials in isolated cat saphenous nerves which were subsequently fixed for light microscopy. They, like Hursh (1939) in his slightly earlier work from the same laboratory, concluded that a scaling factor of 6 was appropriate for large cutaneous afferent fibres. The scaling factor for alpha-efferent fibres was found to be 5.7 in de-afferentated muscle nerves in the cat by Boyd (1964) and the scaling factor for all sizes of gamma-

efferent fibre is 4.5 (Boyd, 1964; Boyd and Davey, 1968; Boyd and Kalu, 1979).

II.ACTION POTENTIAL

Resting membrane potential

In 1902 Bernstein suggested that the resting membrane was selectively permeable to K^+ and that the potential differences across it arose from the tendency of K^+ to move outwards from the more concentrated solution inside a nerve or muscle fibre. Thus if a membrane which is selectively permeable to K^+ separates axoplasm containing 400 mM K^+ from plasma containing 20 mM K^+, one would expect the inside of the membrane to be 75 mV negative to the external solution. This value is obtained from the Nernst equation adopted for potasssium:

$$V_k = RT/ZF \ (\ln [K]_o/[K]_i)$$

where V_k=equilibrium potential of the potassium ion defined in the sense, internal potential minus external potential.

$[K]_o$ and $[K]_i$ are the K^+ concentration outside and inside the fibre. This hypothesis has been substantited by (Keynes and Hodgkin, 1953) in undissected squid axon

with natural circulation, resting membrane potentials of about 70 mV was observed.

2.1 Activation of nervous impulse

Action potentials in peripheral nerve fibre are all or none in amplitude. Action potential can only be elicited when a depolarizing current has reached a certain value, i.e. its threshold. When the depolarizing current changes the resting potential from -60 mv to about -45 mv, an action potential is elicited. A simple way of explaining the reversal of the membrane potential is to assume that when the membrane is activated by an electric current it momentarily becomes selectively permeable to sodium ions. In support of this idea, it is found that the action potential of many but not all excitable tissues disappear in the absence of external sodium or lithium ions. The state of increased sodium conductance wears off after about 1 msec. and the potential therefore tends to return to its original level. The process of repolarization is greatly accelerated by the fact that depolarization causes a delayed rise in the conductance to potassium ions. This speeds up the exit of potassium ions and keeps the whole action potential reasonably short.

The conductance for potassium ion rises and the state of increased potassium ions conductance persists for several msec. Thus the membrane potential approaches

more closely to the potassium equilibrium potential with the result that the fibre undergoes a transient phase of hyperpolarization. Evidence for this suggestion is provided by the observation that the membrane potential during the underswing is affected by potassium concentration which have relatively little effect on the resting membrane potential (Hodgkin and Katz, 1949; Hodgkin and Keynes, 1955b; Frankenhaeuser and Hodgkin, 1956). The resting potential disappears if the external potassium concentration is made equal to the internal concentration and that except at low external concentration of potassium, the potential varies in the manner predicted by the Nernst equation.(Adrian, 1956; Hodgkin and Keynes, 1955b).

2.2 Sequence of event during the action potential, the Voltage clamp method

The development of voltage-clamp method by Cole and Marmont in 1949 facilitated the studies on the electrical properties of the membrane to be more quantitative. In this method, the membrane potential is suddenly displaced from its resting value and held at a fixed potential by a feedback amplifier. The current which flows through a definite area of membrane under the influence of the imposed voltage is measured with a separate amplifier.

When the resting potential of the fibre was raised suddenly by 65 mV. by applying a rectangular pulse to the feedback amplifier which control the membrane potential, the sodium conductance increases greatly and rapidly and result in the movement of sodium ions from the highly concentrated exterior to the interior of cell. When the external solution is made sodium free with the substitution of choline, the inward current pulse is eliminated, thus verifying the inward current is caused by the sodium entry (Hodkgin and Huxley, 1952a).

If the concentration of sodium ion is kept at its normal value and the amplitude of the voltage step is gradually increased, at a certain value, 117 mV in Hodgkin's experiment (Hodgkin et al, 1952), the initial hump of the current disappears. If the early hump of current is carried by sodium ions, the equilibrium potential, at which there is no sodium current, should be given by the Nernst equation:

$$V_{Na} = RT/ZF\ (\ln [Na]_o/[Na]_i)$$

Where V_{Na} is the equilibrium potential of the sodium ion.

$[Na]_o$ and $[Na]_i$ is the sodium concentration outside and inside the fibre.

The results hold to within about 1 mV over a ten-fold range of $[Na]_o$ (Hodgkin and Huxley, 1952).

The sodium current starts to decline at about 0.5 ms and the main current becomes an outward potassium current. This current is uncomplicated by sodium current if the membrane potential during the voltage step is made equal to V_{Na} and this current is not much altered if sodium ions are replaced by choline and is also present in chloride deficient solution. Futher evidences of the outward potassium current include 1. radioactive tracer studies by Hodgkin and Huxley (Hodgkin and Huxley, 1953). 2. the equilibrium potential of the second component varies with the external potassium concentration (Frankenhauser and Hodgkin, 1956).

2.3 Hodgkin - Huxley formulation

The sodium or potassium permeability of a membrane can be expressed in terms of conductance parameters g_{Na} and g_K defined as:

$$g_{Na} = I_{Na} /(V - V_{Na}).$$

$$g_K = I_K /(V - V_K).$$

Where V is the transmembrane potential.

V_{Na} and V_K are the Nernst potentials of sodium and

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potassium ions respectively.

I_{Na} and I_K are the currents per unit area carried by the sodium and potassium ions.

Therefore, the instantaneous value of the current I_{Na} or I_K is directly proportional to the driving force, $V - V_{Na}$ or $V - V_K$.

The rate constants governing the turning on and off of the sodium or potassium conductance depend on temperature, membrane potential and calcium concentration, but are little affected by changes in sodium concentration, or by the direction of the ionic current. (Hodgkin and Huxley, 1952; Frankenhaeuser and Hodgkin, 1957).

A dynamic description of an action potential can be formulated through the use of a membrane model that includes parameters as sodium and potassium conductances, their respective Nernst potential and the membrane capacitance.

The complete expression for the membrane current density I is:

$$I = c\, dV/dt + (V - V_K)\, g_K\, n^4 + (V - V_{Na})\, g_{Na}\, m^3\, h + (V - V_L) g_L$$

Where c = specific membrane capacity per unit area.

g_K is the maximum potassium conductance.

n is the probability that a single particle is in the right place.

g_{Na} is the maximum sodium conductance.

m^3 h = probability that there will be three activating particles and no blocking particle.

g_L = leak conductance.

Relating the current density in a nerve fibre surrounded by a large volume of external fluid is:

$$I = a / 2R\ dV / dX^2$$

Where a = the radius of the axoplasm and R is its resistivity. X is the distance along the nerve. In the case of a fibre propagating at constant velocity (O), X may be replaced by -Ot

Hence:

$$a/2\ RO^2\ d^2\ V/dt^2 = c\ dv/dt + (V - V_k)gK^4\ N + (V - V_{Na})g_{Na}\ m^3\ h + (V - V^L)g^L$$

Impulse propagation

Solution of the Hodgkin - Huxley equation gives the components of membrane conductance during propagated action potential as shown on diagram 1.

The equilibrium potentials of the sodium and potassium ions are represented by the 2 horizontal line. The broken curve gives the membrane potential and the 2 solid curves are the sodium and potassium conductances. At rest, the membrane potential is fairly close to the equilibrium potential of the potassium ion. As the impulse advances, the membrane just ahead of the active regions becomes depolarized by electric currents flowing in a local circuit through the axoplasm and external fluid. The sodium ions enter the fibre owing to the change in membrane potential and rise of sodium conductances. This inward currents make the inside of the fibre positive and provides the current required to depolarize the resting membrane ahead of the active region. At the crest of the spike, the sodium conductance declines and the potassium conductance rises so that the rate of potassium ions leaving the fibre exceeds that of sodium ions enter the fibre. The slow effects of depolarization namely raised potassium conductance and inactivation of the sodium carrying system, persist for a few milliseconds after the spike and gives rise to the refractory period. This refractory period can be divided into absolute and relative refractory period. The transient hyperpolarization are

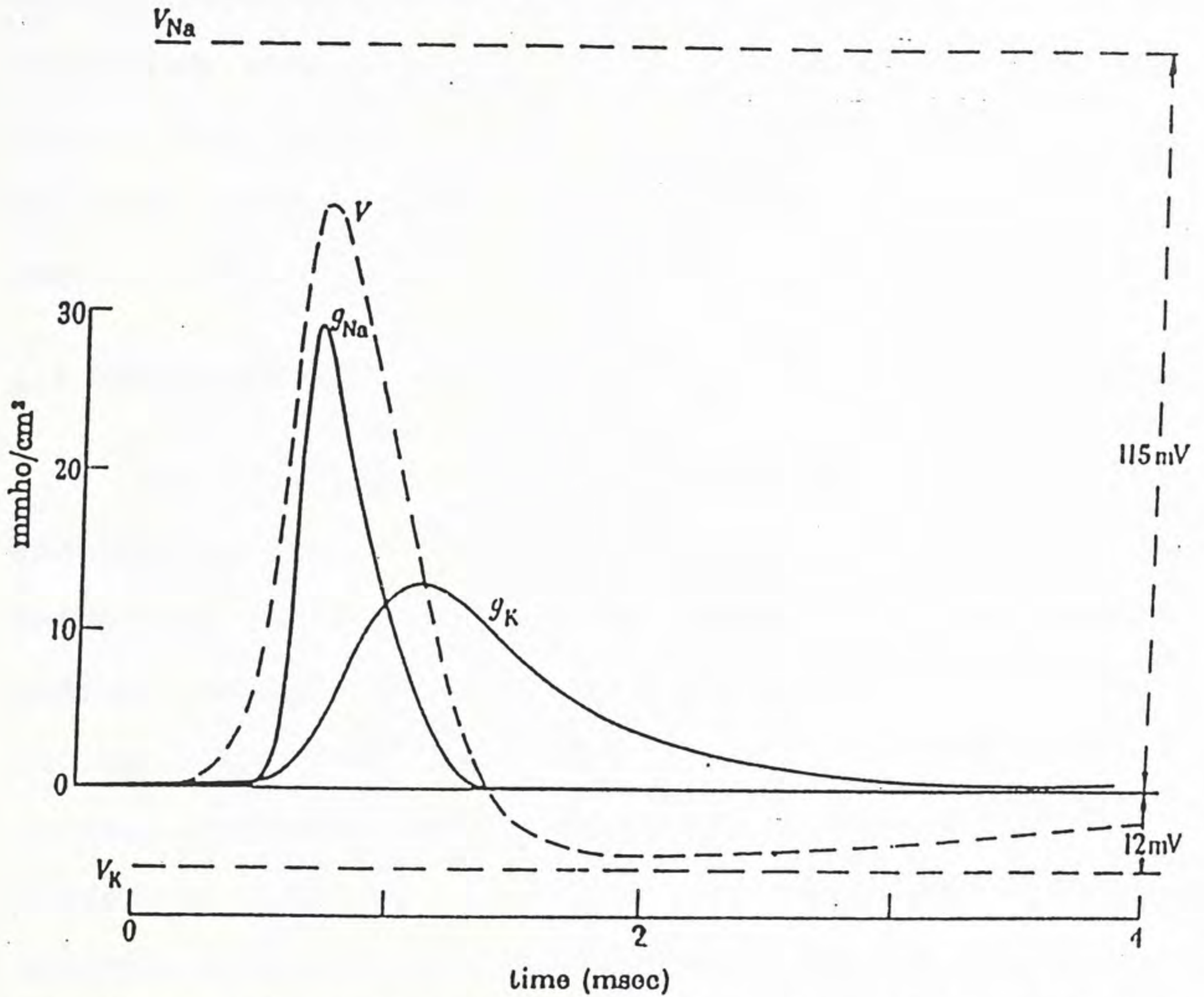

Diagram 1 Theoretical action potential and conductance changes obtained by numerical solution of equation and subsidiary equations for n, m and h; the constants used were appropriate to a temperature of 18·5° C; from Hodgkin & Huxley (1952). Total entry of Na^{+} = 4·33 pmole/cm². Total exit of K^{+} = 4·26 pmole/cm². Velocity = 18·8 m/sec. Temperature = 18·5° C.

caused by the opening of the potassium channels and the inactivation of sodium channels. The residual opening of active potassium channels leads to a greater efflux of potassium from the cell than occurs in the resting state. This efflux causes the membrane potential to hyperpolarize slightly with respect to its normal resting value.

2.4 Extrusion of the sodium ions - sodium pump mechanism

The efflux of the sodium ions can be studied by radioactive tracer method (Hodgkin & Keynes 1955a) in which the nerve fibres were loaded with radioactive sodium ions by stimulation in a solution containing this isotope. The subsequent wash out of this radioactive isotope was collected and measured. In this way, one can study the fate of the sodium ions which enter during activity and are subsequently ejected by the action of a metabolic pump. The energy requirement of the sodium pumping mechanism was confirmed by Hodgkin and Keynes 1955. In their study, dinitrophenol, an metabolic inhibitor was added to a Sepia nerve fibre, the sodium efflux was reduced to about 1/20 of its normal value, the inhibitory effect takes place with a time constant of 15 to 30 minutes and is largely but not completely reversed by washing the dinitrophenol away. Cyanide (1 to 10 mM) and azide (3 mM) have essentially the same action as dinitrophenol on the outflow of sodium. Caldwell (1956) showed that the immediate source of

energy for the pump is by the dephosphorylation of the ATP and that the arginine phosphate acts by replenishing the ATP. Caldwell and Keynes (1957) calculated that about 4 phosphate bonds are broken for each sodium ion expelled. Thus the sodium efflux system is an active process which is energy dependent.

Reabsorption of potassium ions

It was well established that the outflow of sodium and the uptake of potassium depend on metabolism, and the two movement are coupled in a cyclical mechanism (Hodgkin and Keynes, 1955a). Evidence for a coupling between sodium efflux and potassium influx has been obtained in muscle (Keynes, 1954), red cells (Glynn, 1956) and frog skin (Ussing, 1954).

III.AMINOGLYCOSIDE

3.1 Structure of Aminoglycosides

The aminoglycoside group of antibiotics includes a large number of structurally related polycationic compounds containing two or more amino sugars connected by glycosidic linkage to a hexose core. The group consists of streptomycin, neomycin, kanamycin, tobramycin and paromomycin which are derived from different species of Streptomyces; gentamicin, netilmicin and sisomicin which are derived from micromonosporum species, Amikacin is produced through chemical modification of kanamycin (Umezawa, 1974). Neomycin, kanamycin, gentamicin and tobramycin constitute a group distinct from streptomycin. They all contain the base deoxystreptamine linked to various aminohexoses. Streptomycin contains streptidine, neomycin is distinct from others by having three sugar rings rather than two, one of them being the pentose ribose (ring III). It is produced by streptomyces fradiae and was first isolated in 1949 (Waksman and Lechevalier, 1949).

The group are used primarily to treat infection caused by gram negative bacteria. They can act to interfere with protein synthesis in susceptible microorganisms (Pestka, 1971; Sande and Mandell, 1985),

change in cell permeability and transport (Hancock, 1981), or misreading of the genetic code (Pestka, 1971). There are clear differences in the way the various aminoglycosides interact with the protein synthetic machinery at the molecular level. However, the rapid lethal effect can not be explained by the above factors. Schacht (1974, 1976) found that neomycin affects the lipids of the cell membrane instead of inhibiting protein synthesis. Bryan (1984) suggested that the progressive disruption of the cell envelope may explain the lethal action of aminoglycoside antibiotics.

3.2 Antibacterial activity of the aminoglycosides

The aminoglycosides are potent gram negative antimicrobial drugs, kanamycin and gentamicin, tobramycin and amikacin are used most commonly to treat infection caused by aerobic gram negative bacilli. In vitro studies, activity of aminoglycosides are strongly dependent on the cation content and pH of the growth medium. It is because these factors modify the ability of bacteria to actively take up the drugs (Hancock, 1981). Anaerobic bacteria are not susceptible to aminoglycosides because active uptake of these drugs by the bacterium requires respiration (Hancock, 1981).

3.3 Pharmacology of the Aminoglycoside antibiotics

3.3.1 Absorption

Aminoglycosides pass across membranes very poorly and only about 1% of an orally administered dose are absorbed from the gastrointestinal tract (Kunin et al., 1960). Aminoglycosides (principally neomycin and kanamycin) are administered orally to kill the bowl flora before intestinal surgery. Since there is essentially no absorption, this constitutes local administration of the drug. The aminoglycosides (except for neomycin) are routinely administered by intramuscular injection and peak blood level are achieved in about one hour (Leroy, Humbert, Oksunhendler and Fillastre, 1978; Neu, 1982; Ristuccia and Cunha, 1982 and Brogard, Comte and Spach, 1984). Aminoglycosides are presented in many antibiotics formulation used in topical therapy and wound irrigation (MacDonald and Beck 1983). Absorption through intact skin is minimal but there may be significant absorption if aminoglycosides are applied to burned area or open wound.

3.3.2 Distribution

The aminoglycosides are largely excluded from most cells because of their polar nature. The volume of distribution is equal to the extracellular fluid volume (Sande and Mandell, 1985). There is little or no binding of aminoglycosides to serum protiens (Gordon, Regamey and Kirby, 1972). They accumulate to high levels in

kidney where tissue concentration of amikacin and gentamicin have been reported to vary form 16-89 times the plasma concentration (Edwards, 1976). High concentration of aminoglycoside is also found in the endolymph and perilymph of the inner ear (Sande and Mandell 1985).

3.3.3 Excretion

The aminoglycosides are excreted in their active forms by glomerular filtration. Most of the aminoglycosides are not metabolized. The half lives are in the range of 2 to 3 hours (Barza and Scheife, 1977). In anuric individual, the half lives range from 50 to 100 hours or more. The concentration associated with toxicity is not much greater than that required for treatment of many bacterial infection.

3.4 Toxicity of the Aminoglycosides

3.4.1 Neuromuscular blockade

Vital-Brazil and Corrado in 1957 were the first to report that streptomycin causes blockade of the neuromuscular junction in the same way as does curare. Kubikowski and Szrenawski (1963) showed the neuromuscular junction was inhibited by kanamycin in vivo experiment. Vital-Brazil and Prado-Franceschi (1969) showed similar effect by gentamicin. Pittinger et

al. (1970) mentioned in their clinical reports that gentamicin and neomycin increased the anesthetic effect of ether during surgery and caused respiratory paralysis. Neomycin has the blocking effect on the neuromuscular junction in vitro using the nerve and muscle preparation of mouse and rat diaphragm. (Adams and Matthew, 1973; Singh, Harvey and Marshall, 1978; Rosayro and Healy, 1978).

The neuromuscular blocking properties of various aminoglycosides antibiotics such as streptomycin and neomycin have been explained (competitive hypothesis) on the basis that these antibiotics compete with calcium ions for the same receptor on the nerve terminal membrane and produce complexes that are incapable of transmitter release (Vital-Brazil and Prado-Franceschi, 1969; Yamada, Kuno and Iwanaga, 1986)

The neuromuscular blockade produced by the aminoglycosides antibiotics streptomycin and kanamycin and its reversal by calcium were quantitatively studied in the isolated phrenic nerve diaphragm preparation of the rat (Prado, Corrado and Marscillan, 1978). The uptake of calcium with stimulation of the isolated superior cervical ganglion was blocked by neomycin. It decreased the calcium influx associated with the nerve terminal action potential presumably by affecting the voltage sensitivity of the calcium channel (Wright and

Collier, 1977). The neuromuscular block produced by aminoglycoside antibiotics was completely reversed by either calcium ions, 4 aminopyridine or 3, 4 diaminopyridine (Enomoto and Maeno, 1981). McQuillen and Engback (1973) noted that neomycin decreased the probability of release of quanta of acetycholine but not the size of the releaseable store. When administered to cats for 22 - 28 hours, the blockade produced by neomycin came to resemble a curare-induced blockade (Lee and DeSilva, 1979). In addition, postsynatic blocking action of the aminoglycosides had been demonstrated (Vital-Brazil and Corrado, 1957; Singh, Mashall and Harvey, 1982). The postsynaptic effect of two aminoglycoside antibiotics, streptomycin and neomycin were studied on miniature end-plate currents and acetylcholine induced end plate current fluctuations in voltage clamped costo cutaneous muscle of the garter snake. The results suggested that neomycin interacts with the ionic channels of the acetycholine receptor in their open configuration whereas stretpomycin acts primarily by blocking the receptor (Fickers, 1983).

To summarize the available information, neomycin may block neuromuscular junction by a prominent depressant effect on the nerve terminal inhibiting acetycholine release and by a weaker postsynaptic blocking action.

3.4.2 Ototoxicity

Hawkins (1976) defined ototoxicity as the tendency of certain therapeutic agents and other chemical substances to cause functional impairment and cellular degeneration of the tissues of the inner ear, especially of the end organs and nerves of the cochlear and vestibular division of the VIIIth. cranial nerve.

The aminoglycosides are ototoxic to both the hearing and balance functions of the inner ear. Streptomycin primarily affects the vestibular system. Whereas neomycin, kanamycin and amikacin are primarily toxic to the cochlea especially the organ of Corti and Stria Vascularis (Stockhorst and Schacht, 1977) . It has been speculated that the pathogenesis of aminoglycoside ototoxicity is related to the accumulation of the drug in the inner ear fluids; the more ototoxic the aminoglycoside, the longer its retention in the labyrinthine fluids (Federspil et al., 1976). However, study by Tran Ba Huy and coworkers (1986) on the kinetics of gentamacin uptake and release in various organ of the rat show that the uptake of gentamicin by the tissues of the inner ear is dose dependent. It manifests rapid saturation kinetics and leads to a distribution of the drug into different compartments. There is no accumulation either in tissues or in labyrinthine fluids and ototoxicity seems to be related to the penetration of the drug into compartments from

which the half-life of disappearance is extremely low (Tran Ba Huy, Bernard and Schacht, 1986). Recent review by Schacht (1986) stated that aminoglycosides do not accumulate in inner-ear fluids and aminoglycoside level in fluids do not correlate with the otoxic potential of a drug and selective toxicity cannot be explained by selective tissue penetration of the drugs.

The primary pathophysiological leison is destruction of hair cells in the organ of Corti. Light microscope observations of the ototoxic lesion produced by the aminoglycosides antibiotics in the guinea pig cochlea reveal a selective destruction of the end organ sensory hair cells. At low doses, hair cells in the basal turn of the cochlea are affected first, with destruction progressing toward the apex as the aminoglycoside dosage increased. Thus aminoglycoside ototoxicity begins in the region where high frequency sounds are processed and then extends to regions of lower frequency (Brummett, Fox, Bendrick and Himes, 1978; Brummett, 1981).

The extent of the cochlear lesion is determined by the dose and frequency of administration of the drugs and may occur following a latent period after drug treatment has stopped. On an equal-dose basis the ototoxic liability of gentamicin was found to be similar to that of sisomicin, whereas the ototoxic liability of

tobramycin was found to be similar to that of amikacin. Tobramycin and amikacin were less ototoxic than gentamicin and sisomicin (Brummett et al., 1978).

The mechanism of the ototoxic action is not well established. The aminoglycosides do not readily pass across cell membranes and it seems likely that at least the rapid and reversible toxicity may result from an interaction of the drug with superficial membrane structure. Physical studies of neomycin interaction with synaptosome membranes and synthetic lipid monolayers show that physical distortion of membranes occurs at high drug concentration (Lodhi, Weiner and Schact, 1976; 1977). Other probable mechanism includes:

1. Aminoglycosides may have a direct plugging effect on the transduction channels in hair cells. The strong cationic functional groups on the aminoglycosides might enter the transduction channel and block it by "plugging". The blockage is voltage dependent, the reduction of the current become larger with hyperpolarization of the membrane. This has been demonstrated by Ohmori in isolated vestibular hair cells of the chick. (Ohmori, 1985).

2. Aminoglycosides may disturb the normal ionic balance of the endolymph. The disturbance of the normal ionic concentration in the labyrinthine fluid may lead to impairment of electrical activity and nerve conduction.

(Neu and Bendush, 1976).

3. Aminoglycoside may compete with calcium ions for binding sites on membranes necessary for transduction. By increasing calcium concentration in the fluid bathing the apices of hair cells, the effects of aminoglycoside were counteracted (Kroese and Van den Bercken, 1980; Williams, Zenner and Schacht, 1987).

4. Inhibition of ornithine decarbosylase (ODC) activity by the action of neomycin. Neomycin produced a dose-dependent inhibition of ODC with half maximal inhibition observed at 50 uM and almost complete inhibition at 100uM (Henly, Gerhardt and Schacht, 1987).

3.4.3 Relationship between binding of aminoglycosides to polyphosphoinositides and ototoxicity

Schacht (1974, 1976, 1986) found that neomycin affected the lipids of the cell membrane. He also demonstrated a high rate of polyphosphoinositide metabolism in the inner ear. Perfusion of guinea pig cochlea with artificial perilymph containing a high concentration of neomycin causes a rapid reduction in its ability to generate an acoustic current potential in response to a sound stimulus (Nuttall, 1977). Neomycin have been shown to competitively inhibit the binding of radioactive calcium ion to homogenised tissues and to phosphoinositides in artificial tissues (Schacht, 1976).

The suppression effect can be reversed by the application of excess calcium (Sand, 1975; Yanagisawa et al., 1977). Binding of aminoglycosides to phosphatidylinositol has also been demonstrated (Yanagisawa et al., 1984 and Schact, 1986). Neomycin occupies calcium binding sites provided by negatively charged phosphate groups of the polyphosphoinositides in the membrane. The neomycin-bound phosphoinositide is a poor substrate for the dephosphorylating enzyme and the dephosphorylating enzyme and the dephosphorylation phosphorylation cycle is disturbed (Schacht, 1976).

3.4.4 Nephrotoxicity

The aminoglycoside specifically damage the epithelial cells of the renal proximal tubules. This toxic action involves three steps. The uptake of the antibiotics into the cells, the intralysosomal lipid storage and finally cell necrosis. Neomycin is the most nephrotoxic aminoglycoside and streptomycin is the least nephrotoxic. It has been shown that neomycin binds to polyphosphoinositides and inhibits their dephosphorylation in the rat kidney in the same way as in the hair cell (Schtbeci and Schacht, 1977). Recently Hagiwara and his co-worker had shown that the nephrotoxity action of the aminoglycoside takes place by selective inhibition of intracellular protein kinase C which is due to the positive charge contributed by the

amino groups (Hagiwara, Inagaki, Kanamura, Ohta and Hidaka, 1988).

3.4.5 Effects of neomycin on slowly adapting type I (s.a.I) cutaneous mechanoreceptor

Neomycin was found to strongly suppress the excitability of slowly adapting type I (s.a.I) cutaneous mechanoreceptor. A dose of 12.5 mg. of neomycin in 5 min was found to cause strong depression in responsiveness of s.a.I receptors in cat (Baumann, Hamann and Leung 1986). Chronic neomycin injection was found to cause alteration of the mechanical properties of the skin as well as the responsiveness of the s.a.I cutaneous mechanoreceptors in rat. One of the probable mechanism is neomycin may exert its effect on s.a.I receptors by interacting with phosphatidylinositol bisphosphate and inhibit the generation of inositol trisphosphate which had been demonstrated in the hair cells(Baumann et al., 1986; Leung 1986).

VI. POLYPHOSPHOINOSITIDES IN EXCITABLE CELLS

Introduction

The mechanism by which the 'message' generated at the receptor is transferred to the inside of the cell can be divided into 2 classes. One class of receptors mediates its response through formation of cAMP, catalyzed by adenylate cyclase (a protein present on the cytoplasmic side of the membrane). The coupling of adenylate cyclase to the receptor is further regulated by GTP-binding proteins. Another class of receptors mediate its response through calcium mobilization. Evidence is rapidly indicating that a specific class of phospholipids i.e. the phosphoinositides plays an important role in signal transduction from the receptors at the plasma membrane. The link between inositol lipids and calcium signaling was first recognized by Michell in 1975 (Michell, 1975) who noticed that all agonists that seemed to use calcium as an internal signal were also capable of stimulating the hydrolysis of inositol lipids. Detailed biochemcial studies had revealed that agonists seemed to act preferentially to hydrolyze phosphatidylinositol -4,5-bisphosphate (PIP2) to yield inositol 1,4,5 trisphosphate (IP3) and diacylglycerol (DG). These two products appear to function as second messenger in signal transduction in which DG aids in activating protein kinase C and IP3 in elevating the

level of calcium.

Folch in 1949, working with brain lipids, was the first to identify a phosphoinositide fraction which on the basis of chemical analysis appeared to be a 'diphosphoinositide' and Dawson was the first to show how rapidly they were labeled with 32P in comparison with the other phospholipids of brain in 1954, though the chemical structures were still unknown at that time. When more sophisticated chromatographic methodology became available, it was shown that PIP and PIP2 were present in addition to PI (Grado and Ballore, 1961). Polyphosphoinositides probably occur in all eukaryotic cells (Downes and Michell, 1982).

4.1 Polyphosphoinositides and receptor activation

There have been several reviews in recent years dealing mainly with the various aspects of polyphosphoinositide metabolism and its significance in receptor activation (Berridge, 1984; Downes and Michell, 1982; Hawthrone , 1983; Sekin and Hokin, 1986).

The rapid turnover of polyphosphoinositide was first described in brain by Brockerhoff and Ballou in 1962 and in non-neural tissue by Santiago-Cavo and co-workers in 1964. In a brain cytoplasmic particulate

fraction, muscarinic receptor stimulation with acetycholine caused a 30 % loss in PIP. (Santiago-Calvo, Mule, Redman, Hokin and Hokin 1964). Durell and associates (Durell, Sodd and Friedel, 1968) observed the formation of inositol mono-and bisphosphate in synaptosomes in reaction in response to ACh. and they suggested that the primary reaction in response to ACh. was phosphodiesteratic cleavage of PIP and PIP2. In 1977, Abdel-Latif, Akhtar and Hawthorne (1977) showed that muscarinic cholinergic stimulation of rabbit iris muscle caused breakdown of PIP2 within 5 minutes, and this was accompanied by an increase in inositol-1-phosphate (IP), inositol-4-5-bisphosphate (IP2), and inositol-1-4-5-triphosphate (IP3). Despite of the calcium requirement of this response and the ability of a calcium inophore to mimic the above breakdown, the possibility that it might play a role in generating calcium signals was ignored at that time.

The recent revival of interest in polyphosphoinositides began with the finding that calcium mobilizing agonists, such as vasopressin in liver, caused a very rapid breakdown of polyphosphoinositides particularly PIP2 (Creba, Downes, Hawkins, Brewster, Michell and Kirk, 1983; Kirk and Michell, 1981 and Michell, Kirk, Jones, Downes and Creba, 1981). Unlike the effect in iris smooth muscle found by Akhtar's group, the breakdown of

polyphosphoinositides in liver was independent or only partially dependent on calcium (Creba et al. 1983). The rapid break down of polyphosphoinositides had also been confirmed in other tissues such as parotid gland (Weiss, McKinney and putney, 1982), platelets (Billah and Lapetura, 1982; Putney, Burgess, Halenda and McKinney 1983), superior cervical sympathetic ganglia (Bone, Frettern, Palmer, Kirk and Michell, 1984) and hepatocytes (Creba et al., 1983; Kirk and Michell, 1981). The rapid decrease in PIP2 was independent of calcium. The breakdown of polyphosphoinositides in rabbit iris smooth muscle has now been shown to be a secondary effect due to the release of norepinephrine (Akhtar and Abdel-Latif, 1984) and cyclooxygenase products (Rittenhouse, 1984).

It is believed by many that the loss in PI is entirely due to its phosphorylation to polyphosphoinositides rather than direct action of phospholipase C on PI (Agranoff, Murthy and Seguin 1983; Berridge 1983; Michell, Kirk. Jones, Downes and Creba 1981). This is based on the following observations:

1. faster breakdown of polyphosphoinositides as compared to PI,

2. earlier appearance of IP3 and IP2 as compared to IP and

3. an energy requirement to PI disappearance.

Thus the whole sequences of the phosphoinositides and the receptor activation can be summarized as:

1. Interaction of an agonist with its receptor induces the hydrolysis of polyphosphoinositides by the relevant phosphodiesterase enzyme to yield two putative intracellular signals, diacylglycerol and IP3.

2. Resynthesize the lipid precursor so that the whole cycle can be continued.

4.1.1 Calcium gating hypothesis

The link between receptor activation and inositol lipid metabolism was first shown by Hokin and Hokin in 1953. In 1975, Michell noted a correlation between the calcium mobilizing action of certain agonists and the PI effect may be antecedent and causally related to calcium mobilization. The work of Jones and Michell (1974) and Hokin Neaverson (1974) made it clear that the receptor linked reaction was of the phospholipase C type. The substrate at that time being considered to be PI. A loss of this lipid and production of diacylglycerol could be measured chemcially. This led to Michell's gating theory. The support of the calcium gating theory includes the studies of blowfly salivary gland where stimulation with 5-hydroxy-tryptamine (5HT) caused PI loss and the entry of calcium into the epithelial cells

(Fain and Berridge, 1979). When a supramaximal concentration of 5HT was used, this effect was followed by receptor desensitization and abolition of calcium uptake. Incubation of washed glands with myoinositol restored both PI sensitivity to 5HT and calcium transport (Fain and Berridge, 1979). Because of its plasma membrane associates and affinity for calcium, PIP2 is a better candidate for calcium gating than PI (Hawthorne and Pickard, 1979).

In spite of the circumstantial evidence suggesting a role for PI turnover in calcium gating, some of the following points do not neatly fit into this hypothesis:

1. longer time period for the PI effect to be elicited.

2. calcium dependence of the PI effect in certain tissues.

3. the endoplasmic reticulum as the site of most of the PI effect as shown by ^{3}[H] inositol autoradiography and differential centrifugation (Gerber Davies and Hokin, 1973).

It has only been within the past couple of years that a role for phosphoinositides in elevating cytosolic calcium has been established. This has been made possible by permeabilization of cells with detergents or by hypotonicity to prevent entry of added inositol-1-4-5- triphosphate (IP3), an immediate breakdown product of

PIP2. Addition of less than micromolar amounts of IP3 caused a rapid release into the cytosol of 'trigger calcium' apparently from the endoplasmic reticulum and this effect was not mimicked by other inositol phosphates (Streb Irvine, Berridge and Schulz, 1983; Irvine Brown and Berridge, 1984; Hirata, Suematuse, Hasimoto, Hamachi and Koga, 1984).

4.1.2 DIACYLGLYCEROL

Diacylglycerol is the other immediate product of the phosphodiesteratic cleavage of phosphoinositides. A rapid and transient accumulation of DG associated with stimulated phosphoinositides breakdown has been demonstrated in platelets (Haslam and Davidson, 1984; Irvine, 1982; Mauco, Dangelmaier and Smith, 1984; Prescott and Majerus, 1983), mast cells (Igarashi and Kondo, 1980), pituitary cells (Drummond Bushfield and Macphee, 1984; Martin, 1983) and liver (Hughes, Rye, Pickford, Barritt amd Chalmers, 1984; Thomas, Marks, Coll and Willamsian, 1983). Diacylglycerol which is mainly of the 1-stearoyl-2-arachi-donoyl-n-glycerol variety activates a protein kinase distinct from cAMP or cGMP-activated protein kinase. This kinase termed protein kinase C, requires phospholipids and calcium for maximum activity. Its affinity for calcium increases several fold by diacylglycerol (Kishimoto, Takai, Mori and Kikawa, 1980). It appears the tumor-promoting

agents such as phorbol esters can activate at the diacylglycerol site of protein kinase C in intact cells (Kikkawa, Kitano, Saito, Kishimoto, Tamiyama, Tamaka and Nishizuka, 1986).

Thus a stimulus which cause the breakdown of polyphosphoinositides would result in the formation of IP3 and DG. The IP3 branch and the DG branch of the phosphoinositide cascade appear to act synergistically to phosphorylate proteins. The former by elevating intracellular calcium to activate calmodulin-dependent protein phosphorylation and the latter to activate protein kinase C, which phosphorylates a different set of proteins. This synergistic interaction pathway had been demonstrated in different tissues such as platelets (Kaibuchi, Takai, Sawamura, Hoshijma, Fuzikusra and Hishizuka, 1983; Rink, Sanchez and Hallam, 1983), lymphocytes (Mastro and Smith, 1983) and mast cells (Katakami, Kaibuchi, Sawamura, Takai and Nishizuka, 1984).

4.2 Polyphosphoinositides in myelin

Deshmukh et al., (1981, 1982) had shown that rat brain myelin has a active pool of polyphosphoinositides. The greatest incorporation in vivo of ^{32}Pi into these lipids was in a 'heavy myelin' fraction which may contain material from the internal or external mesaxon and membrane loops at the nodes of Ranvier. Both PI and

PIP were more active in this fraction than in the less dense myelin fractions. Polyphosphoinositide phosphomonoesterase was equally active in all fractions. The phospholipase C attacking these lipids was more active in the denser fraction of myelin. The results are consistent with the idea that polyphosphoinositide metabolism is associated with cation flux at the nodes of Ranvier. i.e. involvement with axolemma (Deshmukh, Bear and Brockerhoff 1978; Deshmukh, Kuizon, Bear and Brockerhoff, 1981; 1982).

4.3 Polyphosphoinositides in peripheral nerve

It has been suggested that the lack of inositol and resulting metabolic disturbances are responsible for the nerve conduction defects in diabetes (Winegard, 1983). The concentration of free inositol is reduced in sciatic nerve of streptozotocin-diabetic rats (Greene et al., 1975; Palmano et al., 1977) and in post-mortem nerve samples from diabetic patients (Mayhen et al., 1983). Incubation of sciatic nerve from streptozotocin - diabetic rats with ^{32}Pi for the study of polyphosphoinositide labeling lead to controversial results. Natarajan and his co-workers showed that incubations of isolated sciatic nerves of diabetics rats in a medium containing [^{32}P] orthophosphate gave decreased labeling of phosphatidylinositol and substantial change in the labeling pattern of

phosphatidylinositol phosphate and 4,5 bisphosphate from that of control (Natarajan, Dyck and Schmidt, 1981). Hawthorne (1983) also observed decreased labeling of phosphatidylinositol (Ptd Ins) and a decreased labeling of PIP2 relative to PIP. While Bell and his co-workers (1982) observed increased labeling of PIP2 but no change in Ptd Ins 4P in the nerve from diabetic rats. Whether these changes account for the nerve conduction defect of diabetes need further studies.

4.4 Polyphosphoinositides and the action potential in nerve conduction

There is clear evidence of calcium fluxes as well as the well known sodium and potassium fluxes associated with the action potential in nerve conduction.

Is the polyphosphoinositides metabolism involved in the permeability changes accompanying nerve impulses? Little is known about this possible mechanism. Several papers may be of interest to discuss here:

1. Simmons and his co-workers in 1982 provide evidence that Ptd Ins turnover may regulate sodium / potassium ATPase activity in endoneurium from rabbit vagus nerve. This sodium pump ATPase is a plasma membrane enzyme and its activation by acidic phospholipids has been a controversial subject.

2. Hayashi and his co-workers in 1966 showed that sodium ions stimulated labeling of polyphosphoinositides with ^{32}P in brain slices.

3. Akhtar and Abdel-Latif in 1982 showed that in rabbit iris smooth muscle, acetylcholine stimulated labeling of Ptd Ins and phosphatidate with ^{32}P, and breakdown of polyphosphoinositides in labeled muscle, only in the presence of both sodium and calcium ions. In the absence of sodium ion from the medium, the specific radioactivity of iris muscle. ATP fell to about 1/5 with a corresponding decrease in phospholipid labeling. Abdel-Latif (1983) suggested that muscarinic-induced entry of calcium leads to breakdown of phosphoinositides in the iris muscle cell and this in turn facilitates entry of sodium. Recycling of the phosphoinositides is associated with outflow of sodium (sodium / potassium ATPase). Although this mechanism is fairly speculative, the possible link between sodium pumping and the polyphosphoinositides deserves further study.

4. Hendrickson and Reinertsen (1971) showed that the conversion of triphosphate to diphosphate would result in a release of 70 % bound Ca^{2+} and a 25 % decrease in ligand change. Changes of this magnitude occurring on one side of the nerve axon membrane could conceivably bring about a re-organization of the membrane with a resulting changes in Na^{+} and K^{+} permeability.

5. There are high metabolic activities of the inositides in nerve cell bodies, myelinated as well as poorly myelinated nerve fibres. Stimulation of vagus nerve for 30 minutes increased phosphate incorporation into the phospholipids but the increase has been significant only in the case of IP_3 and IP_2. Tetrodotoxin at concentration sufficient to block transmission had no effect upon phospholipid labeling in vagus or phrenic nerve. Ouabain at blocking concentration did not affect polyphosphoinositide in vagus nerve but increased [^{32}P] labeling of the other phospholipids (White, Schellhase and Hawthorne, 1974).

4.5 Neomycin and the peripheral nerve conduction

The aminoglycosides have been use as tool to study the metabolism of phosphoinositides in signal transduction. It has been used to study the signaling system in a number of tissues (Schibeci and Schacht, 1977; Schacht et al. 1978; Carney, Scott, Gordon and Labelle, 1985; Cockcroft and Gomperts, 1985; Prentki, Deeney, Matschinsky and Joseph, 1986; Schacht, 1986).

The hypothesis put forward in the present study is neomycin may directly affect the nervous conduction by changing the permeability of the Na^+ and / or the K ions or indirectly by affecting the phosphoinositide metabolism in the nervous tissue.

SECTION II:- CONSIDERATIONS IN RELATION TO THE EXPERIMENTAL PROTOCOL

SECTION II:-EXPERIMENTAL CONSIDERATION

1. Interpretation of the Compound Action Potential - Extracellular stimulation and recording.

In 1924, Erlanger and Gasser reported their classical study of motor and sensory conduction in bullfrogs and dogs. They demonstrated the proportional relationship between conduction velocity and diameter of peripheral nerves. Since then, advances had been made to improve the instruments used to measure nerve conduction. Experimental technique had been improved from extracellular recording of whole nerve trunk to single unit extracellular recording by Tasaki and his co-workers (1982).

The nervous activity in the nerve fibre is accompanied by electrical changes which appears across the cell membrane. Such electrical changes can cause electric currents to flow in the cytoplasm of the cell and also in the surrounding conducting fluids. Nervous activity can thus be investigated by either recording the potentials appearing across the membranes or in the extracellular fluids. Conversely by the use of an external source of electromotive force, an electric field can be established through the extracellular fluid, and some of the current which flows can traverse cell membranes affecting the membrane potential and when

the stimulus is high enough i.e. above the threshold of the action potential, action potential can be elicited.

In extracellular recording, two electrodes are placed in the extracellular fluid surrounding the nerve fibres. The recorded potential is the potential difference in the extracellular fluid between the two regions where the electrode make their contact. When two closely spaced electrodes lying side by side along a line parallel to the longitudinal axis of the axon, an biphasic action potential is obtained.

Extracellular recording has its advantages of being easy to operate and without causing any traumatization to the intracelluar structure. They are particular useful in long terms experiments and simultaneous multilead recordings from different structures.

The magnitude of the extracellularly recorded signal depends on a number of factors which include the distance between the neural membrane (axolemma) and the recording electrodes and the electrical properties of the interposing non-neural tissue and the surrounding medium. In compound action potential, it is a algebraic sum of the contribution from each fibres. A resultant signal in terms of millivolts in amplitude can usually be obtained.

2. The use of frog sciatic nerve for the investigation of Action Potential.

The sciatic nerve of the frog had once been the most frequent material used by physiologists for investigating excitability phenomena. Sciatic nerve is selected here for the pilot study mainly because it is relatively easy to dissect and the results can be compared with those from other workers.

3.The choice of rat-tail for in vivo study of conduction of nervous impulses.

The results obtained from the in vitro study of the compound action potential of the frog sciatic nerve cannot be compared with the living mammalian species. Moreover, the original design of the experimental set-up does not allow an accurate detection of the C-potential. It is because the stimulation sites and recording sites are too close together making it difficult to disperse the A potential from the C potential. Therefore, the next step of the experiment involves in vivo study of mammalian nerve fibres. The coccygeal nerve roots which supply the rat tail is selected in this experiment on the account that it is easier to measure the exact conduction distance as the course of the nerve is relatively straight and a conduction distance of 10 cm. can easily be obtained.

4.Morphology of the rat-tail muscles

The rat-tail is innervated from six pairs of dorsal and ventral coccygeal roots. One spinal segment usually innervates four muscular segments. Outside the vertebral column the roots join to form one dorsomedial and one ventrolateral longitudinal nerve trunk on each side. The two dorsal nerve trunks supply the dorsal muscles and the skin at the dorsal aspect aspect of the tail. The lateral and ventral muscles and skin region get their innervation from branches of the ventrolateral longitudinal nerve trunks. The innervation to the dorsal and ventral muscles are mixed nerve and contain skin nerve fibres branching off from the muscle nerves (Stag, 1964).

5. Ionic permeabilities of the perineurium

The perineurium forms a continuous multilayer sheath around the fascicles of peripheral nerves. The fascicles are in turn surrounded by a connective sheath - the endoneurium. The perineurium composes of a variable number of concentric layers of thin flat cells on both sides with collagen fibrils interspersed between the layers of cells (Low, 1976). The adjacent cells of a layer and sometimes cells of adjacent layers are held together by intercellular tight junctions (zonulal occludentes) and a sparse number of desmosomes. These

morphological feature contribute to the diffusion barrier properties of the perineurium to small ions (Krnjeviv, 1954). The endoneurial space which surrounds the axons of peripheral nerves is delineated by the perineurium and endoneurial vasculature, these two structure constitute blood nerve barrier. Just as the blood-brain barrier regulates the immediate fluid environment of the elements of the central nervous system, the blood-nerve barriers controls the micro-environment of the peripheral axons.

Recent studies by Weerasuriya and his coworkers had shown that there was no evidence to suggest active transperineurial transport of sodium ion or potassium ion the paracellular routes of transperineurial permeation of sodium , potassium chloride ions and [^{14} C] sucrose exhibited a size-dependent permselectivity to these solutes.(Weerasuriya, Rapoport and Taylor, 1980; Weerasuriya and Rapport, 1986; Weerasuriya,1987). In view of the above findings. A desheathed nerve is necessary in order to facilitate the diffusion of ions into the axon.

SECTION III:- METHODOLOGY

SECTION III:- METHODS

1. In vitro study of conduction of A compound Action Potential in frog sciatic nerve

1.1. Preparation of frog sciatic nerve

Leopard frog (Rana pipiens) of either sex were used in this study. The frog was paralyzed by physical destruction of the central nervous system by the double-pithing method. The skin was cut around the body at the abdominal region and was then pulled downwards and off the lower part of the body and legs. It was then laid on its ventral surface on a paraffin board. The sciatic nerve was identified which lies between the semitendinosus and semimembranosus muscle. It was then separated carefully from the surrounding connective tissue with forceps to pull on adjacent tissues and the hooked glass rod to lift gently the nerve. A pair of fine scissors was used to free the connective tissue from the nerve especially where the nerve passes through the knee joint. At all stages, the nerve was kept moist by wetting with Ringer solution at room temperature of 20°C. The sciatic nerve between the upper part of thigh and lower one-third of the calf region was then dissected out. It was then transferred to a Ringer solution bath bubbled with 95% oxygen and

5% carbon dioxide at room temperature of 20°C.

1.2 Preparation of the desheathed nerve fibre.

The nerve was desheathed by a longitudinal incision through the epineurium layer with a sharp dissecting knife. The sharp knife blade was prepared by breaking the edge of a double sided carbon razor blade (Eagle brand, China) with a pair of pointed pliers. The blade was examined under the microscope to ensure that the point edge was sharp enough. An 'Eclipse' pin vice was used to fix the blade and acted as a holder. The two ends of the nerve were tied with thread so as to facilitate placement of the nerve onto the nerve chamber at the next stage.

A plastic chamber, as shown on diagram 2, was pierced by silver/silver chloride electrodes spaced at prearranged distances was made. The chamber was separated into 3 parts by 2 partitions which was connected by 2 round holes which allowed subsequent passage of the nerve. An inlet which was made from a hypodermic needle was arranged in the middle chamber so as to allow constant bubbling of 95% oxygen and 5% carbon dioxide. The nerve was passed alternating above and below each electrode from one to other. Good contact of the nerve with the electrode was ensured by allowing a little tension applied onto the thread which tied the

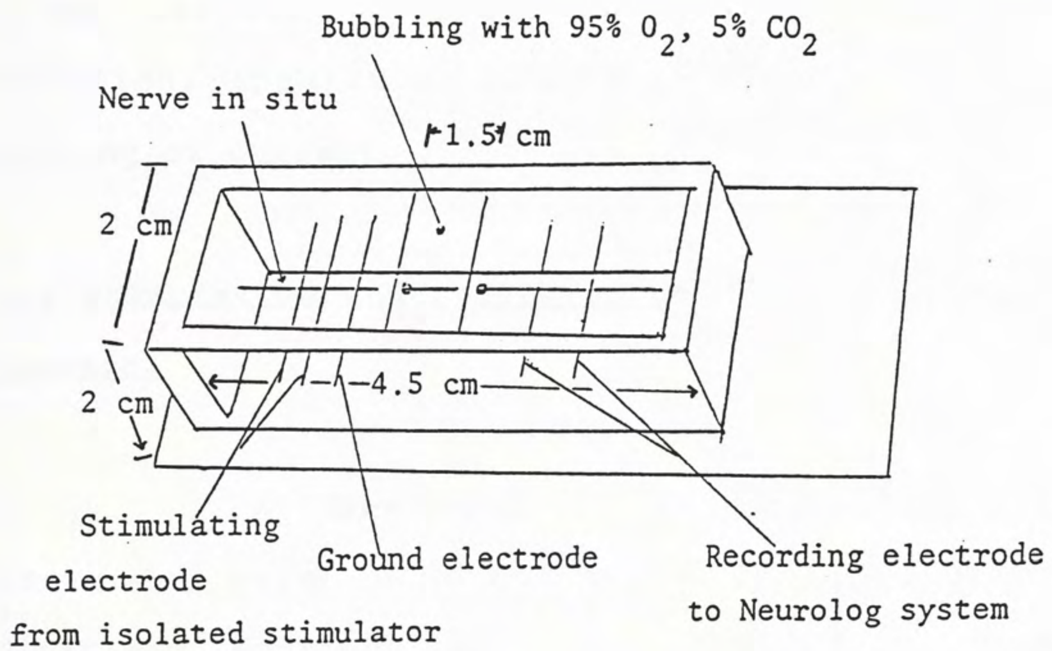

Diagram 2 In vitro study of conduction of
A coumpound action potential in Frog sciatic nerve
- EXPERIMENTAL SET-UP -

two end of the nerve. Agar solution was used to fill the holes so that the three chambers are separated from each other. The middle chamber was either filled with Ringer solution, neomycin in Ringer solution or 20 mM KCL in isotonic Ringer solution. The other two chambers were filled with liquid paraffin to prevent drying of the nerve, isolate it from the air and acted as an electrical insulating medium to reduce any possible shunting of current.

1.3 Stimulation and recording of the A-compound Action Potential

At one end of the nerve, stimulating electrodes were connected to an isolated stimulator (Digitimer, model DS 2). A pair of recording electrodes was connected to a differential amplifier (Neurlog, NL 103). A ground electrode was placed between the two pairs of electrodes.

The desheathed sciatic nerve was stimulated at one end with electrical stimulus from the stimulator. The duration of the stimulus was maintained at 0.06 ms Threshold of the A compound action potential was determined by gradually increasing the stimulus strength. The threshold voltage required to elicit an action potential was recorded. Supramaximal stimulation for the A fibres was then given. A train of eight

stimuli with intervals of 1 second was then given. An interval of one minute was allowed between each set of stimuli. After a stable compound action potential was established, the Ringer solution was then replaced either by Ringer solution with neomycin (3500 mg/l) or 20 mM KCL in isotonic Ringer solution. The resultant osmolarities were in the range of 221-225, 224-231 and 221-225 mOsm/kg. respectively. Change of solution was made with a tube system attached to a roller pump. The electrical stimulation was continued for the next 30 minutes and recorded signals were amplified by the Neurolog amplifier system.

The compound action potential in response to the electrical stimulation were amplified with a differential amplifier (Neurolog 103) (100x), filtered (Neurolog NL 125) averaged (Neurolog Nl 750) and displayed on a storage oscilloscope (Tektronix 5130N). The differential amplifier was used to minimize the interference and the balance, the potentiometer on the amplifier, was adjusted to minimize common mode signals such as mains interference or shock artifacts present in the recording and to avoid any DC background which would affect the averaged compound action potential. Ground level were adjusted prior to the recording of the action potential. The differential amplifier was used to minimize the interference. The bandwidth of the filter

was set from 60 Hz. to 1.5 kHz. The averaged compound action potential was recorded on a single channel chart recorder (Graphtec miniwriter model WTR 771A) and was later used for calculation of the conduction velocities and the amplitude of the action potential. The conducting distance was measured with a caliper between the stimulating and recording electrodes.

1.4 Preparation of the Ringer solution.

Danforth and Helmreich (1964) Frog ringer solution was used:

NaCl	83 mM
KCl	2 mM
$CaCl_2$	2.5 mM
$MgCl_2$	3 mM
$NaHCO_3$	25 mM
KH_2PO_4	1.2 mM
D-glucose	5.5 mM
Total	122.2 mM

The solution was bubbled with 95 % O_2 and 5 % CO_2. pH value was maintained at 7.45 ± 0.05.

1.5 Preparation of the Neomycin (3500 mg/l) in Ringer solution.

Neomycin sulphate (Mycifradin, Upjohn) was used in this study. Stock solution was made by dissolving 350 mg of neomycin with 10 ml of Ringer solution. Each ml of

the stock solution was mixed with 9 c.c of Ringer solution. The concentration of the final neomycin solution would thus be 3500 mg/l or 127.9 mM.

pH of the solution was maintained at 7.45 ± 0.05 and bubbled with 95 % O_2, 5 % CO_2.

1.6 Preparation of 20 mM KCL in isotonic Ringer solution.

Composition of 20 mM KCl in isotonic Ringer solution:

NaCl	65 mM
KCl	20 mM
$CaCl_2$	2.5 mM
$MgCl_2$	3 mM
$NaHCO_3$	25 mM
KH_2PO_4	1.2 mM
D-glucose	5.5 mM
Total	122.2 mM

pH was maintained at 7.45 ±0.05 and bubbled with 95 % O_2, 5 % CO_2.

1.7 Data and statistical analysis

The latency as well as the peak amplitude were determined on the chart recorder. For the determination of the conduction velocity of the A potential, it is assumed that the acute effect of neomycin would only take place in the portion of the nerve which was actually immersed with neomycin solution. Any change in latency was presumed due to the effect on the immersed portion.

The relative change in the amplitude and the conduction velocity were also determined. This was done in each individual experiment. The amplitude and the conduction velocity recorded before the application of neomycin or KCL were taken as 100 % . Any change in amplitude or conduction velocity was relative to a change in this value.

Statistical analysis of the conduction velocity and the average amplitude was carried out using the oneway analysis of variance. The null hypothesis was that there was no significant difference between the control and the treated period. The significant level was set at $P \leq 0.05$. Statistical analysis was performed by the statistical package SPSS/PC (Statistical Package for the Social Science). The results of the data were expressed as mean $\pm$ standard deviation of mean.

To test the reliability of the experimental set

up, oneway analysis of the control group was performed. The null hypothesis was there was no significant difference in the amplitude and the conduction velocity between each period of time.

2. In vivo study of conduction of action potential in rat ventrolateral tail nerve

2.1 General preparation

Sprague-Dawley rats of either sex of body weight about 350g were used in this study. The animals were anesthetized with urethane (20% w/v. 6 ml/kg i.p.), (Sigma, U.S.A.). Supplementary doses of about 0.5 ml were given i.p. if necessary. One carotid artery was cannulated for monitoring of arterial blood pressure. The mean blood pressure was normally at 100 mm Hg. The body temperature was kept at 38°C by a thermostatically controlled electric blanket (Bioscience, CFP-8185).

The rat was laid on its dorsal surface. The rat tail was enclosed by a U-shaped glass tube with warm water continuously circulated around it by a Watson Marlow peristaltic pump (model 502 S). The water was kept warm at 38°C by a water bath which was thermostatically set at 38°C . Two subcutaneous thermistor probes were placed on the proximal and distal end of the rat-tail to monitor the subcutaneous temperature throughout the experiment.

The ventrolateral tail nerve which supplied the lateral and ventral muscles was used in this experiment. Three incision sites of about 2.5 cm each

were made in the scrotal, middle and distal region of the tail. The incisions were made slightly lateral so as to avoid any possible damage to the blood vessel which lies in the central region of rat-tail. Threads were then fixed to the skin around the incision sites for the attachment of the skin flap to a ring system of screws to form pool which to be filled with liquid paraffin (Riedel de Haen, DABB) or Krebs solution.

2.2 Surgical procedures

The ventrolateral tail nerve lies underneath the tendon units. Some of the tendon units had to be removed for adequate exposure of the nerve. Care had to be taken here to avoid any damaged to the nearby blood vessels. The dissection was carried out under a dissecting microscope (Wild, Model M 650), (Magnification 6-40x). The connective tissue around the nerve was freed from the nerve trunk with two fine forceps and was subsequently dissected with a pair of fine iris scissors. A hooked glass rod was used to gently lift the nerve so as to free the nerve from the surrounding tissue. At all stages, the pool was bathed with Krebs solution. The freeing of the nerve was then repeated in the other 2 sites. Desheathing of the nerve was performed in the central pool which was subsequently exposed to Krebs solution or other test solution. The nerve was desheathed by a longitudinal incision through

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the epineurium layer with a sharp dissecting knife. The preparation of the sharp knife has been described in the previous section. The dissection was carried out under the dissecting microscope with magnification of 40x. Any tissue fluid and blood which accumulated after the dissection was removed by using Pasteur pipettes and small pieces of lint cloth. The proximal and distal pool was then filled with warm liquid paraffin at about 34 ^{o}C to prevent drying of the nerve, to isolate it from the air and to act as an insulating medium to reduces any possible shunting of the circuit. The middle pool was filled with either Krebs solution, 10 mM K_2SO_4 in Krebs solution or Krebs solution with neomycin (3500 mg/l). The solution was infused into the pool through a syringe pump (Harvard apparatus) at a rate of 0.5 ml per min. The solution was kept warm by running through a chamber with circulating water of 38°C. The solution in the pool was constantly carried away by a roller pump system (Perista pump SJ - 1211).

The electrode placement, screw ring system and the U-shape glass tube are shown in diagram 3 and 4 respectively.

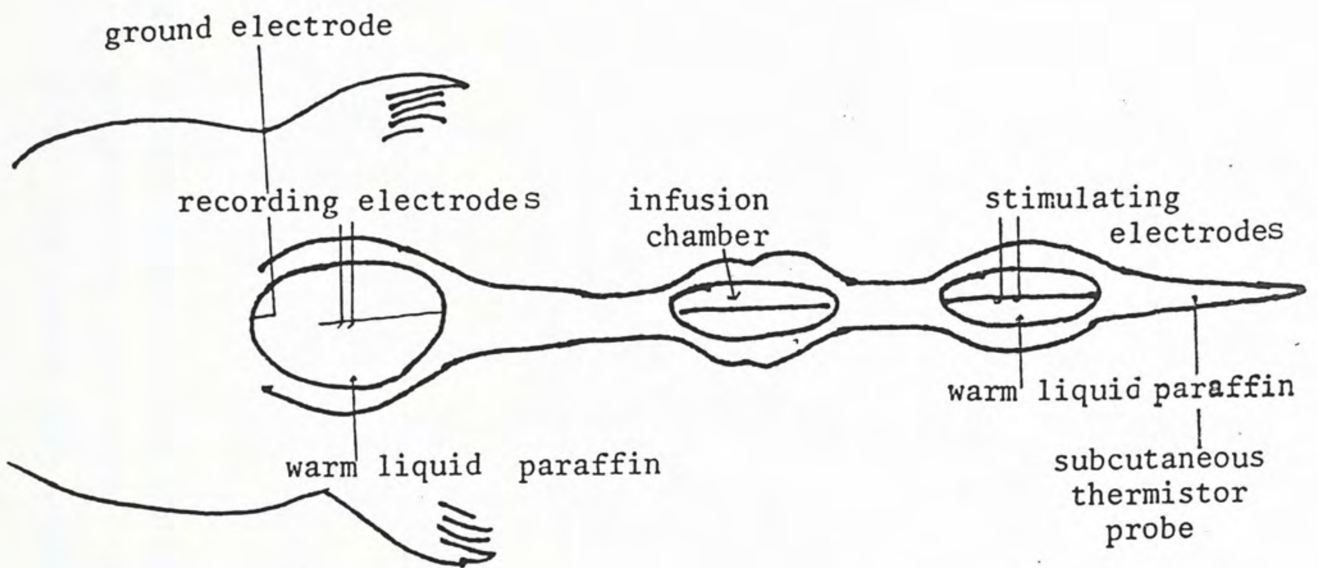


Diagram 3 In vivo study of conduction of A and C potential in rat ventrolateral tail nerve - electrode placement.

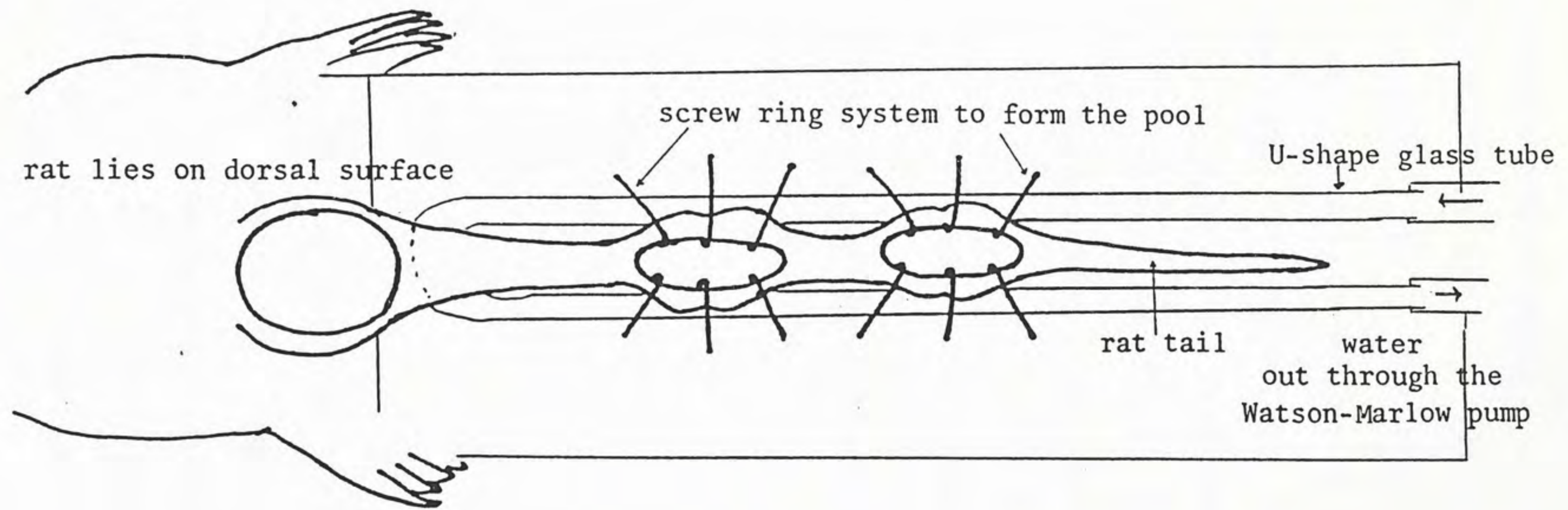


Diagram 4 In vivo study of conduction of A and C potential in rat ventrolateral tail nerve - Experimental set-up showing screw ring system and U shape glass tube.

2.3 Stimulation and recording of A and C compound Action Potential

Bipolar silver-silver chloride hook electrodes were used as stimulating and recording electrodes. Two micro-manipulators (Prior, England) were used to allow three dimensional fine adjustment of the electrode onto the nerve fibre. The stimulating electrode was placed at the caudal end so that muscle contraction was minimized. The stimulating voltage was supplied by an insulated stimulator (Digitimer, Model DS2) and was triggered by the Digitimer (D4030). The recording electrode was connected to a differential amplifier (Neurolog NL103). A pointed silver wire which acted as the ground electrode was inserted into the surrounding muscle to work as an earthing electrode.

Electrical stimulation was given distally, the duration of each stimulus was maintained at 0.1 and 1 ms respectively for A and C potential. A square wave form of impulse was given. Threshold of the A compound action potential was first observed. C compound action potential was subsequently detected by gradually increasing the stimulus strength. The stimulus strength required to elicit the A or C potential was recorded. Supramaximal stimulation for the A or C fibres were then given. A train of stimulation which consisted of ten stimuli were given. A rest period of 10 s was given

between each stimulus. An interval of 5 minutes was allowed between the trains of stimuli. Either the A or C potential was recorded throughout one set of experiment.

The A or C compound action potential in response to the electrical stimulation were amplified with differential amplifier (Neurolog NL 103), filtered, (Neurolog NL 125), and further amplified (NL 106 AC-DC amplifier). The balance and ground level was adjusted and the recording electrodes were positioned carefully so as to get the least possible ground noise or background DC level. The differential amplifier was used to amplify the incoming signal and minimize the interference. The amplification was 1000x. The filter was set to a bandwidth of 60 Hz. to 1.5 kHz.

The original recordings of the nervous impulses from the differential amplifier were stored with a four channel FM tape recorder (Hewlett Packard 3946A). One channel was used for the recording of the nervous impulses while one direct channel was used to record the synchronized trigger pulses from the digitimer D4030 which control the stimulator. The direct channel also recorded the voice which described the particulars of each set of stimuli. Tape speed was set at (3 3/4" per s.). The nervous potential from the differential amplifier were also converted into digital signal by the CED 1401 AD convertor to a 8087 based microcomputer and stored on floppy diskettes for later data analysis. The

instantaneous potential as well as the averaged potential were displaced on a storage oscilloscope (Tektronix 5130N). Averaged potential was recorded on a two channel chart recorder (Miniwriter WTR 751). The conducting distance was measured with a caliper between the stimulating and recording electrodes.

After a stable A or C potential was established, Krebs solution with neomycin (3500 mg/l) was infused into the pool at an infusion rate of 0.5 ml per min. Trains of stimuli as described were continued for the next 60 minutes and recordings were made with the Neurolog amplifier system. After one hour of exposure to neomycin solution, the nerve was washed with Krebs solution with a infusion rate of 0.5 ml per min. Trains of stimuli as described were given in the next 30 minutes. Compound action potentials were recorded to see if there was any change with the Krebs solution. After 30 minutes, the solution was changed to 10 mM K_2SO_4 which gave 20 mM of K^+ ions in the Krebs solution. When complete blocking of the potential was established, the nerve was then flushed with Krebs solution to see the reversibility of the effect of hyperkalemia.

2.4 Preparation of the Krebs solution

The Krebs and Henseleit formula was used (Burton 1975)

NaCl	117 mM
KCl	4.7 mM
$CaCl_2$	2.5 mM
$MgSO_4$	1.2 mM
$NaHCO_3$	24.8 mM
KH_2PO_4	1.2 mM
D-glucose	11.1 mM
Total	162.5 mM

The osmolarity was in the range of 294-300 mOsm/kg and the solution was bubbled with 95 % O_2 and 5 % CO_2. pH was maintained at 7.35$\pm$ 0.05.

2.5 Preparation of Neomycin solution in Krebs solution

Neomycin sulphate (Mycifradin, Upjohn) was used in this study. Stock solution was made by dissolving 350 mg of neomycin with 10 ml of Krebs solution. Each ml of the stock solution was mixed up with 9 ml. of Krebs solution. The concentration of the final neomycin solution would thus be 3500 mg/l or 168.2 mM. and the osmolarity was 297-303 mOsm/Kg.

pH was maintained at 7.35 $\pm$0.05 and the solution was bubbled with 95% O_2, 5% CO_2.

2.6 Preparation of 10 mM K_2SO_4 in Krebs solution.

Composition of the solution:

NaCl	97 mM
KCl	4.7 mM
$CaCl_2$	2.5 mM
$MgSO_4$	1.2 mM
$NaHCO_3$	24.8 mM
KH_2PO_4	1.2 mM
K_2SO_4	10 mM
D-glucose	11.1 mM
Total	152.5 mM

pH was maintained at 7.35 ± 0.05 and the solution was bubbled with 95% O_2, 5% CO_2.

3 DATA STORAGE

The nervous potential from the differential amplifier were converted into digital signals with the CED 1401 AD convertor. The data storage computer program required 4k bytes of memory for each of data or stimulation. A typical waveform of 250 msec will require a 2500 sample points i.e 10 sample points were allocated to each msec which gave a fine resolution of change in amplitude in every msec. Data storage included the automatic determination of the baseline which was done by sampling the ground noise level before the stimulation. Data were also stored on magnetic tape for later retrieval.

3.1 MAGNETIC TAPE

A Hewlett Packard 3964A four channel tape recorder was used. One FM channel was used for the recording of the nervous impulse. The channel was calibrated to give an output signal of the same amplitude as the input signal. The original nervous impulses from the recording electrode were stored on tape after amplification by 1000x by the differential amplifier. The direct channel was used to record the synchronized trigger pulses from the digitimer D4030 which controlled the stimulator. It also recorded the voice which described the particulars of each set of stimuli.

3.2 DATA ANALYSIS

The data analysis program allows retrieval of the original potential on screen and can determine the latency, peak potential, full wave rectification and integration of the potential. The latent period of the A and C potential can be traced on screen by the cursor. The accuracy of the cursor movement was $\pm$0.1 ms. Therefore, the accuracy of the latent period was within 0.1 ms. For the A potential, the earliest peak i.e. the fastest conduction velocity was located by the cursor. Latency was shown on screen as well as on the printer. In the C potential, the shortest and longest latent period were located on screen by the cursor and was subsequently shown on screen and the printer. Besides the latent period, the range of either A or C potential was located by the cursor and full wave rectification of the whole range was performed. Integration of these range divided by the interval would thus give the average amplitude of the A and C potential respectively. Since integration of the range would inevitably involved the ground noise, determination of the baseline or zero line was necessary before the actual integration. This was performed by the data analysis program which was done by sampling the ground noise level before the stimulation. In the data storage program, 10 sample

points were allocated to each ms. Therefore, a fine resolution of change in potential in every ms. can be achieved.

For the determination of the conduction velocity of A and C potential, It is assumed that the acute effect of neomycin would only be taken place in the portion of the nerve actually immersed with neomycin solution. Any change in latency was the result of the effect at the immersed portion.

The relative change in the amplitude and the conduction velocity were also determined. This was done in each individual experiment. The amplitude and the conduction velocity recorded before the application of neomycin or K_2SO_4 were taken as 100 % . Any change in amplitude or conduction velocity was a relative change to this value.

3.3 STATISTICAL ANALYSIS

Statistical analysis of the conduction velocity and the average amplitude was carried out using the oneway analysis of variance. The null hypothesis was that there was no significant difference between the control and the treated period. The significant level was set at $P \leq 0.05$. Statistical analysis was performed by the statistical package SPSS/PC (Statistical Package for the Social Sciences). The results of the data were expressed as mean $\pm$ standard deviation of mean.

SECTION IV:- RESULTS

RESULTS:-

STIMULATION PARAMETERS

1.1 FROG SCIATIC NERVE:- CONTROL STUDIES

In the control studies, six frogs of both sexes were used. The mean value threshold value to elicit a compound action potential was 0.47 $\pm$0.11 V. The stimulating voltage was the supramaximal stimulus above which further increase in stimulus will not lead to any increase in amplitude and changes of the conduction velocity. Mean value of the stimulating voltage in this series of experiments was 4 $\pm$0.9 V. The stimulation duration was fixed at 0.06 ms. The temperature of the nerve chamber throughout the experiment was 20.5 $\pm$0.5 ^{o}C. pH of the Ringer solution was within the range of 7.43 $\pm$ 0.01. Details of the data with the minimum, maximum, mean and standard deviation are listed in Table 1.

NEOMYCIN STUDIES

Fourteen frogs of both sexes were used . Mean value of the threshold was 0.5 $\pm$ 0.13 V. The mean stimulating voltage was 4.8 $\pm$ 1.6 V. Stimulation duration was again fixed at 0.06 ms. The mean temperature inside the nerve chamber was 20.6 $\pm$0.8 ^{o}C. pH was within the range of 7.42$\pm$ 0.01. Details of the data with the minimum, maximum, mean and standard deviation are listed in Table 2.

20 mM KCL IN ISOTONIC RINGER SOLUTION

6 frogs of both sexes were used. Mean value of the threshold was 0.47 $\pm$ 0.1 V. Mean value of the stimulating voltage was 4.6 $\pm$ 2.4 V. Stimulation duration was again fixed at 0.06 ms. Mean temperature inside the nerve chamber was 20.7 $\pm$0.5 ^{o}C. pH value was within the range of 7.42 $\pm$0.1. Details of the data with the minimum, maximum and standard deviation are listed in Table 3.

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF THRESHOLD, STIMULATING VOLTAGE AND DURATION
TEMPERATURE AND pH VALUE

MODE	THRESHOLD in V.	DURATION in msec	STIMULATING VOLTAGE in V.	TEMP. in Celsius	pH value

TABLE 1:- CONTROL

MODE	THRESHOLD in V.	DURATION in msec	STIMULATING VOLTAGE in V.	TEMP. in Celsius	pH value
Minimum	0.3	.06	3.0	20.0	7.42
Maximum	0.6	.06	5.0	21.0	7.43
Mean	0.475	.06	4.0	20.5	7.43
StdDev	0.117	0.00	.9	.5	.01
N	6	6	6	6	6

TABLE 2:-NEOMYCIN IN ISOTONIC RINGER SOLUTION

MODE	THRESHOLD in V.	DURATION in msec	STIMULATING VOLTAGE in V.	TEMP. in Celsius	pH value
Minimum	0.16	.06	3.0	20.0	7.41
Maximum	0.70	.06	9.0	22.0	7.44
Mean	0.511	.06	4.8	20.6	7.42
StdDev	0.138	0.00	1.6	.8	.01
N	14	14	14	14	14

TABLE 3:-20 mM KCL IN ISOTONIC RINGER SOLUTION

MODE	THRESHOLD in V.	DURATION in msec	STIMULATING VOLTAGE in V.	TEMP. in Celsius	pH value
Minimum	0.35	.06	2.4	20.0	7.41
Maximum	0.70	.06	9.0	21.0	7.43
Mean	0.475	.06	4.6	20.7	7.42
StdDev	0.117	0.00	2.4	.5	.01
N	6	6	6	6	6

1.2RAT VENTROLATERAL TAIL NERVE

A COMPOUND ACTION POTENTIAL

Six rats of both sexes with weights of about 350 g were used. Mean value of the threshold was 0.3 ±0.2 V. Mean stimulating voltage was 3.4 ±1.6 V. Stimulus duration was kept at 0.1 msec. The mean conductive distance which is the distance between the stimulation electrode and the recording electrode was 9.1 ±0.8 cm. The temperature recorded at the caudal part of the rat-tail was 33°C. pH of the Krebs solution, neomycin and 10 mM K_2SO_4 Krebs solution were within the range of 7.36 ±0.02, 7.32 ±0.03 and 7.37 ±0.02 respectively. The details of the data with the minimum, maximum, mean and standard deviation are listed in Table 4.

1.3 C-POTENTIAL

Nine rats of both sexes with weights of about 350 g were used. The minimum voltage required to elicit the C potential was taken as its threshold. The mean threshold voltage was 1.4 ±0.6 V. Any potential with conduction velocity below 2.5 m/s was classified as C potential (Gasser, 1950). The stimulating voltage was defined as the supramaximal stimulus in which further increase in stimulus would not lead to any increase in the amplitude and the conduction velocity of the C

potential. The stimulating voltage required in this series of study was 14 $\pm$3 V. Stimulating duration was kept at 1 ms. The average conductive distance was 10.0 $\pm$0.8 cm. Mean temperature was 33 $\pm$1 ^{o}C. pH of Krebs solution, neomycin and 10 mM K_2SO_4 Krebs solution were within the range of 7.34 $\pm$0.05, 7.33 $\pm$0.02 and 7.34$\pm$ 0.02 respectively. Details of the data with minimum, maximum, mean and standard deviation are listed in Table 5.

TABLE 4

A COMPOUND ACTION POTENTIAL

RAT VENTROLATERAL TAIL NERVE

DATA OF THRESHOLD, STIMULATING VOLTAGE AND DURATION

TEMPERATURE AND pH VALUE

	THRESHOLD in V.	STIMULATING VOLTAGE in V.	CONDUCTIVE DISTANCE in cm	TEMP. in Celcius	pH KREBS SOLUTION	pH NEOMYCIN	PH POTASSIUM SULPHATE
Minimum	.1	1.0	8.5	33	7.35	7.30	7.35
Maximum	.5	5.0	10.2	33	7.40	7.35	7.40
Mean	.3	3.4	9.1	33	7.36	7.32	7.37
StdDev	.2	1.6	.8	0	.02	.03	.02
N	6	6	6	6	6	6	6

TABLE 5

C COMPOUND ACTION POTENTIAL

RAT VENTROLATERAL TAIL NERVE

DATA OF THRESHOLD, STIMULATING VOLTAGE AND DURATION

TEMPERATURE AND pH VALUE

	THRESHOLD in V.	STIMULATING VOLTAGE in V.	CONDUCTIVE DISTANCE in cm	TEMP. in Celsius	pH KREBS SOLUTION	pH NEOMYCIN	PH POTASSIUM SULPHATE
Minimum	1.0	10	8.3	33	7.30	7.30	7.30
Maximum	3.0	20	10.9	35	7.40	7.35	7.37
Mean	1.4	14	10.0	33	7.34	7.33	7.34
StdDev	.6	3	.8	1	.03	.02	.02
N	9	9	9	9	9	9	9

AMPLITUDES AND CONDUCTION VELOCITIES

OF

A AND C COMPOUND ACTION POTENTIAL

2.1 FROG SCIATIC NERVE

AMPLITUDES OF A COMPOUND ACTION POTENTIAL:-CONTROL STUDIES

Table 6 lists the minimum, maximum, mean and standard deviation of the potential of 6 frogs sciatic nerves. When the potential was stabilized, stimulation was given every minute for 30 minutes. The change in amplitude was plotted against time. Figure 1 represents the changes in amplitudes with time. Results were expressed as mean $\pm$SEM. The mean value at the beginning of the treatment was 2.92 $\pm$0.45 mV. and the mean value at the end of treatment was 3.04 $\pm$ 0.47 mV. There was no significant difference between the changes in amplitude with the time ($p > 0.05$).

When the value obtained from the first stimulus was taken as 100%, the change in amplitudes of the potential is shown in figure 2.

CONDUCTION VELOCITIES

Table 7 lists the minimum, maximum, mean and standard deviation of the conduction velocities of 6 frogs sciatic nerves. The change in conduction

velocities was plotted against time. Figure 3 represents the change of the conduction velocíties with the time. Results were expressed as mean ±SEM. The mean value at the beginning of the treatment was 21.8 ± 1.2 m/s. and the mean value at the end of treatment was 21.9 ± 1.1 m/s. There was no significant difference between the change in conduction velocity with time ($p > 0.05$). When the value obtained from the first stimulus was taken as 100%, the change in conduction velocities is shown in figure 4.

TABLE 6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
CONTROL STUDIES

TIME in minutes	AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
0		
Minimum	1.70	98.18
Maximum	4.32	100.00
Mean	2.90	99.69
StdDev	1.08	0.74
N	6	6
5		
Minimum	1.70	98.18
Maximum	4.32	100.00
Mean	2.90	99.69
StdDev	1.08	0.74
N	6	6
10		
Minimum	1.70	98.18
Maximum	4.32	100.00
Mean	2.90	99.69
StdDev	1.08	0.74
N	6	6
15		
Minimum	1.70	100.00
Maximum	4.40	100.00
Mean	2.92	100.00
StdDev	1.10	0.00
N	6	6
16 (1)		
Minimum	1.70	100.00
Maximum	4.40	100.00
Mean	2.92	100.00
StdDev	1.10	0.00
N	6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
CONTROL STUDIES

TIME in minutes		AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
17	(2)		
Minimum		1.70	100.00
Maximum		4.40	100.00
Mean		2.92	100.00
StdDev		1.10	0.00
N		6	6
18	(3)		
Minimum		1.70	100.00
Maximum		4.40	101.67
Mean		2.93	100.28
StdDev		1.11	.68
N		6	6
19	(4)		
Minimum		1.70	100.00
Maximum		4.40	101.67
Mean		2.93	100.28
StdDev		1.11	.68
N		6	6
20	(5)		
Minimum		1.70	100.00
Maximum		4.40	101.67
Mean		2.93	100.28
StdDev		1.11	.68
N		6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
CONTROL STUDIES

TIME in minutes	AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
21 (6)		
Minimum	1.70	100.00
Maximum	4.40	101.92
Mean	2.94	100.60
StdDev	1.11	.93
N	6	6
22 (7)		
Minimum	1.67	98.24
Maximum	4.40	103.85
Mean	2.96	100.95
StdDev	1.13	2.54
N	6	6
23 (8)		
Minimum	1.64	96.47
Maximum	4.40	103.85
Mean	2.92	99.74
StdDev	1.13	2.90
N	6	6
24 (9)		
Minimum	1.64	96.47
Maximum	4.40	103.85
Mean	2.92	99.74
StdDev	1.13	2.90
N	6	6
25 (10)		
Minimum	1.64	96.47
Maximum	4.40	106.15
Mean	2.93	100.13
StdDev	1.13	3.62
N	6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
CONTROL STUDIES

TIME in minutes		AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
26	(11)		
Minimum		1.64	96.47
Maximum		4.47	106.15
Mean		2.95	100.98
StdDev		1.13	3.16
N		6	6
27	(12)		
Minimum		1.70	100.00
Maximum		4.47	106.15
Mean		2.97	101.57
StdDev		1.12	2.38
N		6	6
28	(13)		
Minimum		1.70	100.00
Maximum		4.47	107.69
Mean		2.97	101.82
StdDev		1.12	2.98
N		6	6
29	(14)		
Minimum		1.70	98.00
Maximum		4.47	107.69
Mean		2.96	101.49
StdDev		1.11	3.32
N		6	6
30	(15)		
Minimum		1.70	98.00
Maximum		4.47	107.69
Mean		2.97	101.82
StdDev		1.12	3.43
N		6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
CONTROL STUDIES

TIME in minutes	—	AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
31	(16)		
Minimum		1.70	98.00
Maximum		4.47	107.69
Mean		2.98	102.09
StdDev		1.13	3.67
N		6	6
32	(17)		
Minimum		1.70	98.00
Maximum		4.47	110.38
Mean		2.99	102.54
StdDev		1.13	4.55
N		6	6
33	(18)		
Minimum		1.70	98.00
Maximum		4.47	110.38
Mean		2.99	102.54
StdDev		1.13	4.55
N		6	6
34	(19)		
Minimum		1.70	100.00
Maximum		4.47	110.38
Mean		3.01	102.88
StdDev		1.13	4.21
N		6	6
35	(20)		
Minimum		1.70	100.00
Maximum		4.47	110.38
Mean		3.01	102.88
StdDev		1.13	4.21
N		6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
CONTROL STUDIES

TIME in minutes		AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
36	(21)		
Minimum		1.70	100.00
Maximum		4.47	110.38
Mean		3.01	102.88
StdDev		1.13	4.21
N		6	6
37	(22)		
Minimum		1.70	100.00
Maximum		4.47	110.38
Mean		3.01	102.88
StdDev		1.13	4.21
N		6	6
38	(23)		
Minimum		1.70	100.00
Maximum		4.47	112.69
Mean		3.03	103.64
StdDev		1.14	4.84
N		6	6
39	(24)		
Minimum		1.70	100.00
Maximum		4.47	112.69
Mean		3.02	103.26
StdDev		1.13	5.05
N		6	6
40	(25)		
Minimum		1.70	100.00
Maximum		4.47	112.69
Mean		3.03	103.64
StdDev		1.14	4.84
N		6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
CONTROL STUDIES

TIME in minutes	AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
41 (26)		
Minimum	1.70	100.00
Maximum	4.47	112.69
Mean	3.03	103.64
StdDev	1.14	4.84
N	6	6
42 (27)		
Minimum	1.70	100.00
Maximum	4.47	112.69
Mean	3.03	103.64
StdDev	1.14	4.84
N	6	6
43 (28)		
Minimum	1.70	100.00
Maximum	4.47	112.69
Mean	3.04	103.92
StdDev	1.15	5.00
N	6	6
44 (29)		
Minimum	1.70	100.00
Maximum	4.47	112.69
Mean	3.04	103.92
StdDev	1.15	5.00
N	6	6
45 (30)		
Minimum	1.70	100.00
Maximum	4.47	112.69
Mean	3.04	103.92
StdDev	1.15	5.00
N	6	6

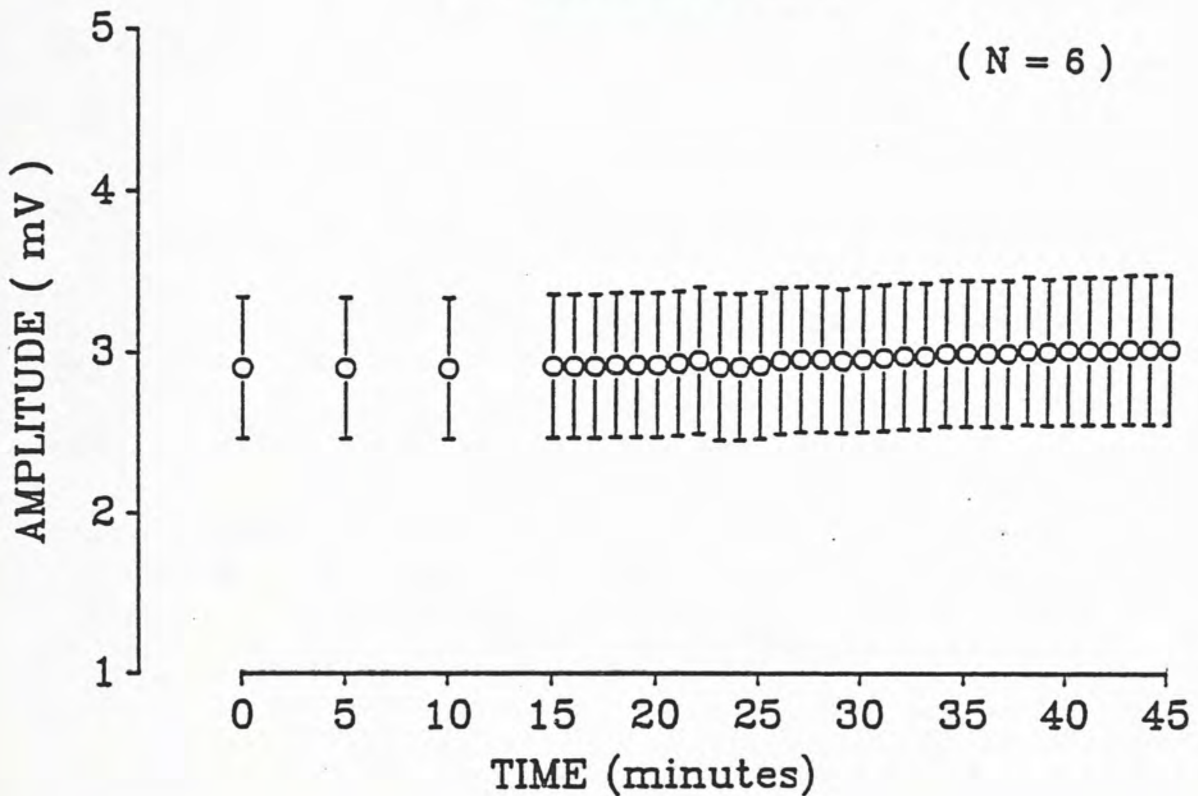


Figure 1 are the data of A compound action potentials of 6 frog sciatic nerves treated with Ringer solution for 45 minutes. Stimulation was given every minute from 15 minutes onward. Peak amplitude was plotted against the time . Results were expressed as mean $\pm$SEM.

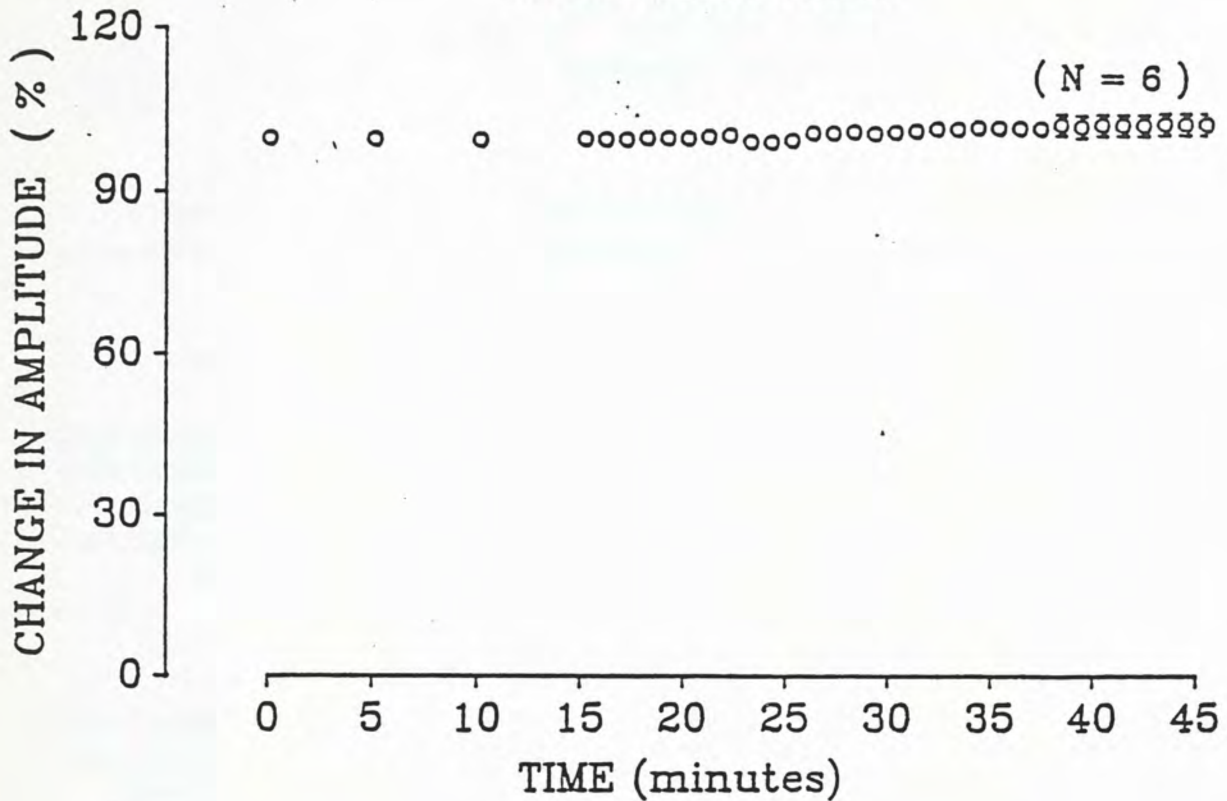


Figure 2 are the data of A compound action potentials of 6 frog sciatic nerves treated with Ringer solution for 45 minutes. Stimulation was given every minute from 15 minutes onward. Change in amplitude was plotted against time. Results were expressed as mean ±SEM.

TABLE 7

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
CONTROL STUDIES

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
0		
Minimum	19.6	100.00
Maximum	26.0	104.35
Mean	22.1	100.72
StdDev	3.0	1.78
N	6	6
5		
Minimum	19.6	100.00
Maximum	26.0	104.35
Mean	22.1	100.72
StdDev	3.0	1.78
N	6	6
10		
Minimum	18.8	100.00
Maximum	26.0	100.00
Mean	21.8	100.00
StdDev	2.9	0.00
N	6	6
15		
Minimum	18.8	100.00
Maximum	26.0	100.00
Mean	21.8	100.00
StdDev	2.9	0.00
N	6	6
16 (1)		
Minimum	18.8	100.00
Maximum	26.0	100.00
Mean	21.8	100.00
StdDev	2.9	0.00
N	6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
CONTROL STUDIES

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
17 (2)		
Minimum	18.8	100.00
Maximum	26.0	100.00
Mean	21.8	100.00
StdDev	2.9	0.00
N	6	6
18 (3)		
Minimum	18.8	100.00
Maximum	27.4	105.53
Mean	22.4	102.46
StdDev	3.1	2.73
N	6	6
19 (4)		
Minimum	18.8	100.00
Maximum	26.4	105.76
Mean	22.2	101.64
StdDev	3.2	2.60
N	6	6
20 (5)		
Minimum	18.8	96.33
Maximum	27.4	105.53
Mean	22.1	101.17
StdDev	3.3	3.53
N	6	6
21 (6)		
Minimum	18.8	100.00
Maximum	27.4	105.76
Mean	22.5	102.74
StdDev	3.5	3.01
N	6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
CONTROL STUDIES

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
22 (7)		
Minimum	18.8	100.00
Maximum	26.4	105.76
Mean	22.4	102.50
StdDev	3.0	2.79
N	6	6
23 (8)		
Minimum	18.8	100.00
Maximum	26.0	105.13
Mean	22.0	100.85
StdDev	2.8	2.09
N	6	6
24 (9)		
Minimum	18.8	100.00
Maximum	26.0	105.13
Mean	22.1	101.53
StdDev	2.7	2.40
N	6	6
25 (10)		
Minimum	18.8	100.00
Maximum	26.0	100.00
Mean	21.8	100.00
StdDev	2.9	0.00
N	6	6
26 (11)		
Minimum	18.8	100.00
Maximum	26.0	100.00
Mean	21.8	100.00
StdDev	2.9	0.00
N	6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
CONTROL STUDIES

TIME in minutes		CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
27	(12)		
Minimum		18.8	100.00
Maximum		26.0	105.13
Mean		22.0	100.85
StdDev		2.8	2.09
N		6	6
28	(13)		
Minimum		18.8	100.00
Maximum		26.0	103.88
Mean		21.9	100.65
StdDev		2.9	1.59
N		6	6
29	(14)		
Minimum		18.8	100.00
Maximum		26.0	100.00
Mean		21.8	100.00
StdDev		2.9	0.00
N		6	6
30	(15)		
Minimum		18.8	100.00
Maximum		26.0	100.00
Mean		21.8	100.00
StdDev		2.9	0.00
N		6	6
31	(16)		
Minimum		18.8	100.00
Maximum		26.0	110.53
Mean		22.2	101.75
StdDev		2.8	4.30
N		6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
CONTROL STUDIES

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
32 (17)		
Minimum	18.8	100.00
Maximum	26.0	105.13
Mean	22.0	100.85
StdDev	2.8	2.09
N	6	6
33 (18)		
Minimum	18.8	100.00
Maximum	26.0	105.13
Mean	22.0	100.85
StdDev	2.8	2.09
N	6	6
34 (19)		
Minimum	19.4	100.00
Maximum	26.0	105.13
Mean	22.1	101.40
StdDev	2.7	2.24
N	6	6
35 (20)		
Minimum	19.4	100.00
Maximum	26.0	103.24
Mean	21.9	100.54
StdDev	2.8	1.32
N	6	6
36 (21)		
Minimum	18.8	96.33
Maximum	26.0	105.13
Mean	21.9	100.24
StdDev	2.9	2.81
N	6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
CONTROL STUDIES

TIME in minutes		CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
37	(22)		
Minimum		19.4	96.33
Maximum		26.0	105.13
Mean		22.0	100.78
StdDev		2.7	3.05
N		6	6
38	(23)		
Minimum		19.4	96.33
Maximum		26.0	105.13
Mean		22.0	100.78
StdDev		2.7	3.05
N		6	6
39	(24)		
Minimum		19.4	96.33
Maximum		26.0	105.13
Mean		22.0	100.78
StdDev		2.7	3.05
N		6	6
40	(25)		
Minimum		19.4	96.33
Maximum		26.0	105.13
Mean		22.0	100.78
StdDev		2.7	3.05
N		6	6
41	(26)		
Minimum		19.4	96.33
Maximum		26.0	105.13
Mean		22.0	100.78
StdDev		2.7	3.05
N		6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
CONTROL STUDIES

TIME in minutes		CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
42	(27)		
Minimum		19.4	96.33
Maximum		26.0	105.13
Mean		22.0	100.78
StdDev		2.7	3.05
N		6	6
43	(28)		
Minimum		19.4	96.33
Maximum		26.0	105.13
Mean		22.0	100.78
StdDev		2.7	3.05
N		6	6
44	(29)		
Minimum		19.4	96.33
Maximum		26.0	105.13
Mean		22.0	100.78
StdDev		2.7	3.05
N		6	6
45	(30)		
Minimum		19.4	96.33
Maximum		26.0	103.24
Mean		21.8	99.93
StdDev		2.8	2.19
N		6	6

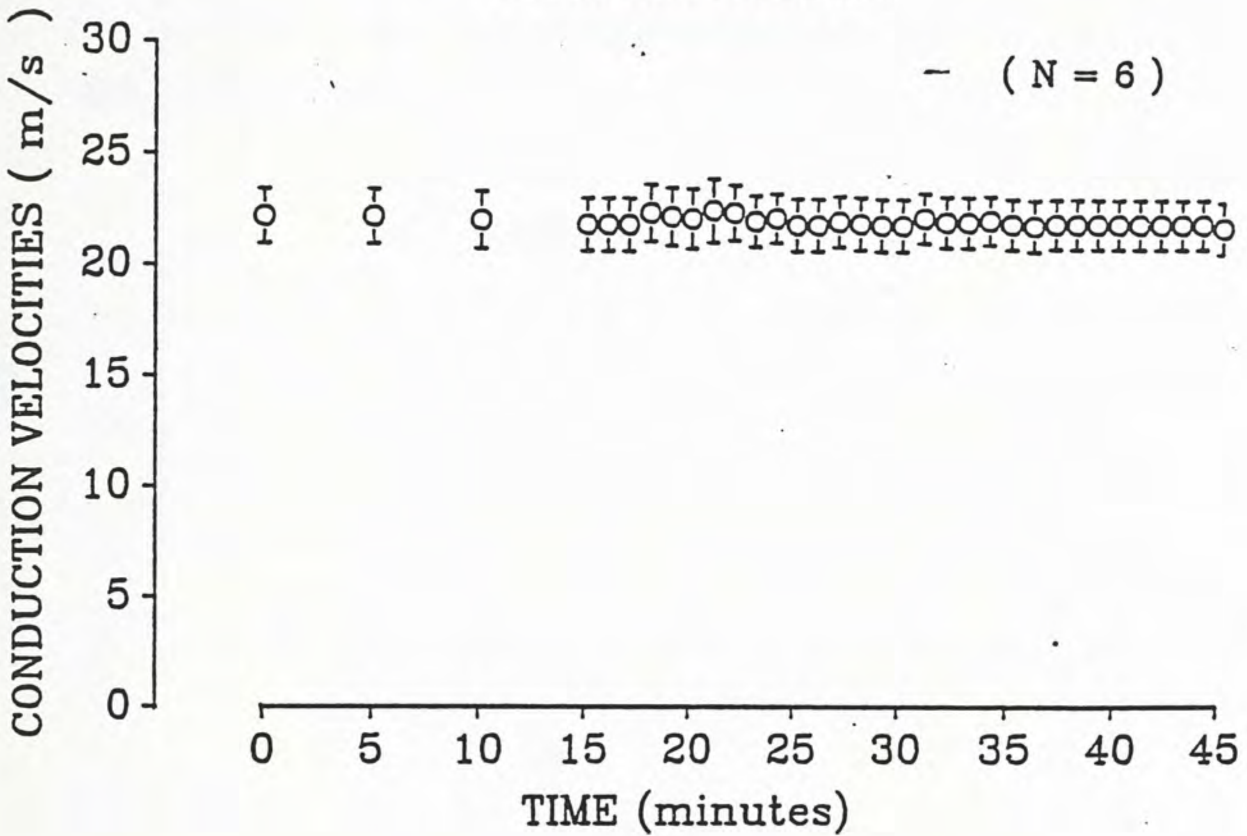


Figure 3 are the data of A compound action potentials of 6 frog sciatic nerves treated with Ringer solution for 45 minutes. Stimulation was given every minute from 15 minutes onward. Conduction velocity was plotted against time. Results were expressed as mean $\pm$SEM.

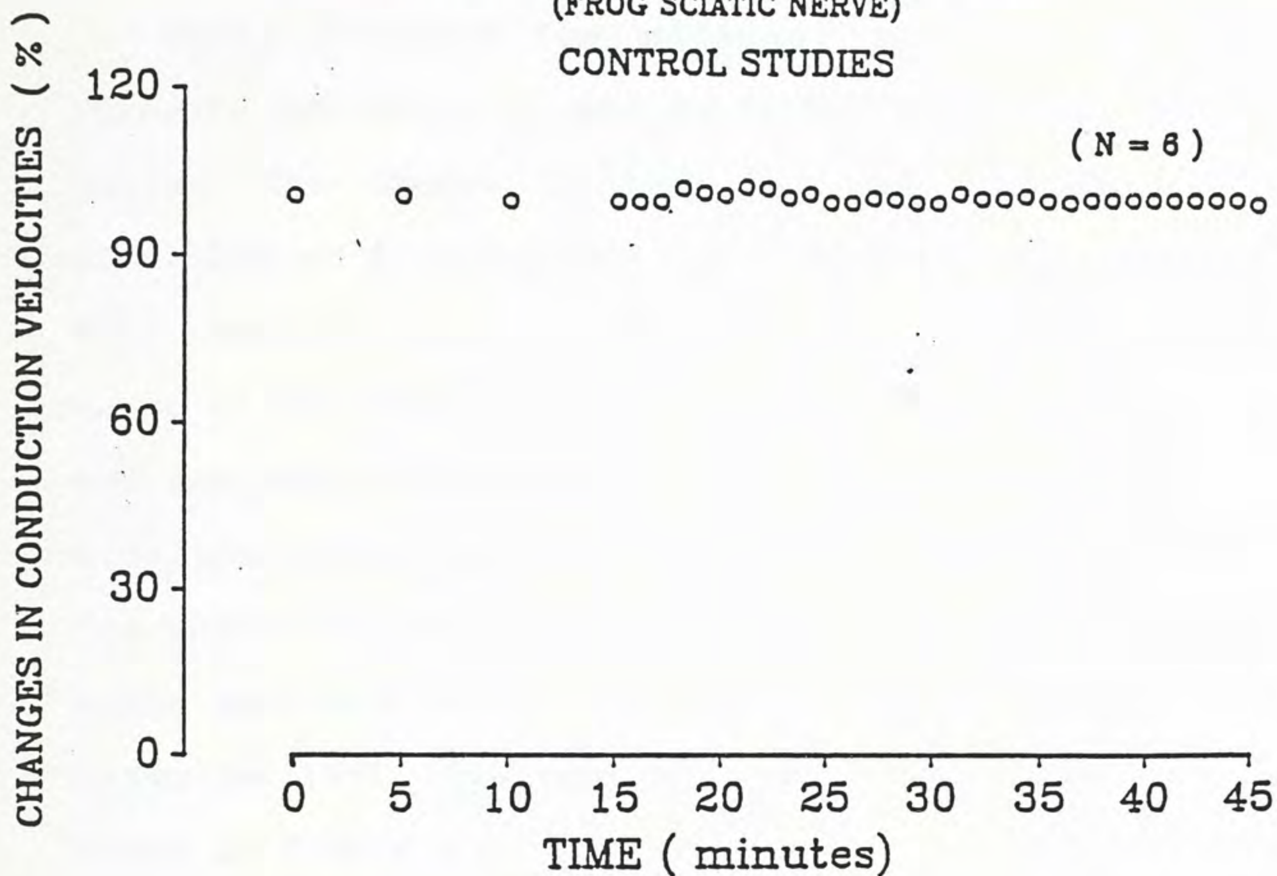


Figure 4 are the data of A compound action potentials of 6 frog sciatic nerves treated with Ringer solution for 45 minutes. Stimulation was given every minute from 15 minutes onward. Change in conduction velocity was plotted against the time. Results were expressed as mean $\pm$SEM.

NEOMYCIN

AMPLITUDES OF COMPOUND ACTION POTENTIAL

Table 8 lists the minimum, maximum, mean and standard deviation of the potential of 14 frog sciatic nerves. The change in amplitude was plotted against time. Figure 5 represents the change in amplitude with time. Results were expressed as mean $\pm$ SEM. The mean value at the beginning of treatment was 2.55 $\pm$0.29 mV. and the mean value at the end of treatment was 2.57 $\pm$ 0.29 mV. There was no significant difference between the change in amplitude with time ($p > 0.05$). When the value obtained before the application of neomycin was taken as 100%, the change in amplitude with time is shown in figure 6.

CONDUCTION VELOCITIES

Table 9 lists the minimum, maximum, mean and standard deviation of the conduction velocities of 14 frog sciatic nerves. The change in conduction velocity was plotted against time. Figure 7 represents the change in conduction velocity with time. Results were expressed as mean $\pm$SEM. The mean value at the beginning of the treatment was 21.6 $\pm$0.5 m/s and the mean value at the end of the treatment was 21.5 $\pm$0.4 m/s. There was no significant difference between the change in amplitude with time ($p > 0.05$). When the value obtained from the first stimulus was taken as 100%, the change in conduction velocity is shown in figure 8.

TABLE 8

A COMPOUND ACTION POTENTIAL

FROG SCIATIC NERVE
DATA OF AMPLITUDE
NEOMYCIN STUDIES

TIME in minutes	AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
0		
Minimum	1.12	93.33
Maximum	4.70	102.85
Mean	2.52	99.06
StdDev	1.05	2.72
N	14	14
5		
Minimum	1.12	93.33
Maximum	4.86	103.40
Mean	2.53	99.53
StdDev	1.08	2.82
N	14	14
10		
Minimum	1.12	93.30
Maximum	4.70	103.13
Mean	2.55	100.13
StdDev	1.08	2.46
N	14	14
15 (Neomycin started)		
Minimum	1.20	100.00
Maximum	4.70	100.00
Mean	2.55	100.00
StdDev	1.08	0.00
N	14	14
16 (1)		
Minimum	1.20	100.00
Maximum	4.70	100.00
Mean	2.55	100.00
StdDev	1.08	0.00
N	14	14

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
NEOMYCIN STUDIES

TIME in minutes		AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
17	(2)		
Minimum		1.20	100.00
Maximum		4.70	101.64
Mean		2.55	100.12
StdDev		1.08	.44
N		14	14
18	(3)		
Minimum		1.20	100.00
Maximum		4.70	101.79
Mean		2.55	100.24
StdDev		1.08	.62
N		14	14
19	(4)		
Minimum		1.20	97.78
Maximum		4.70	101.64
Mean		2.55	99.96
StdDev		1.08	.76
N		14	14
20	(5)		
Minimum		1.20	97.78
Maximum		4.70	105.00
Mean		2.55	100.32
StdDev		1.08	1.55
N		14	14
21	(6)		
Minimum		1.20	97.78
Maximum		4.70	105.00
Mean		2.55	100.18
StdDev		1.07	1.66
N		14	14

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
NEOMYCIN STUDIES

TIME in minutes		AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
22	(7)		
Minimum		1.20	97.78
Maximum		4.70	105.00
Mean		2.55	100.30
StdDev		1.07	1.72
N		14	14
23	(8)		
Minimum		1.20	97.78
Maximum		4.70	105.00
Mean		2.55	100.30
StdDev		1.07	1.72
N		14	14
24	(9)		
Minimum		1.20	97.78
Maximum		4.70	105.00
Mean		2.55	100.16
StdDev		1.06	1.82
N		14	14
25	(10)		
Minimum		1.17	97.50
Maximum		4.70	105.00
Mean		2.55	100.16
StdDev		1.08	1.87
N		14	14
26	(11)		
Minimum		1.15	95.83
Maximum		4.70	108.00
Mean		2.56	100.47
StdDev		1.08	3.02
N		14	14

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
NEOMYCIN STUDIES

TIME in minutes		AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
27	(12)		
Minimum		1.15	95.83
Maximum		4.70	108.00
Mean		2.55	100.20
StdDev		1.07	2.97
N		14	14
28	(13)		
Minimum		1.15	95.83
Maximum		4.70	108.00
Mean		2.55	100.06
StdDev		1.07	3.03
N		14	14
29	(14)		
Minimum		1.15	95.83
Maximum		4.70	108.00
Mean		2.55	99.92
StdDev		1.07	3.16
N		14	14
30	(15)		
Minimum		1.15	95.83
Maximum		4.70	108.00
Mean		2.56	100.34
StdDev		1.08	3.01
N		14	14
31	(16)		
Minimum		1.15	95.83
Maximum		4.70	105.00
Mean		2.56	100.26
StdDev		1.09	2.89
N		14	14

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
NEOMYCIN STUDIES

TIME in minutes	AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
32 (17)		
Minimum	1.17	96.11
Maximum	4.70	105.00
Mean	2.56	100.52
StdDev	1.09	2.65
N	14	14
33 (18)		
Minimum	1.15	95.83
Maximum	4.70	105.00
Mean	2.55	100.12
StdDev	1.08	2.85
N	14	14
34 (19)		
Minimum	1.12	93.33
Maximum	4.70	105.00
Mean	2.56	100.22
StdDev	1.09	3.19
N	14	14
35 (20)		
Minimum	1.15	95.83
Maximum	4.70	105.00
Mean	2.56	100.26
StdDev	1.09	2.89
N	14	14
36 (21)		
Minimum	1.15	95.83
Maximum	4.70	108.20
Mean	2.57	100.62
StdDev	1.10	3.41
N	14	14

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
NEOMYCIN STUDIES

TIME in minutes		AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
37	(22)		
Minimum		1.12	93.33
Maximum		4.70	108.20
Mean		2.57	100.58
StdDev		1.09	3.74
N		14	14
38	(23)		
Minimum		1.12	93.33
Maximum		4.70	108.20
Mean		2.57	100.58
StdDev		1.09	3.74
N		14	14
39	(24)		
Minimum		1.15	95.83
Maximum		4.70	108.20
Mean		2.57	100.77
StdDev		1.09	3.40
N		14	14
40	(25)		
Minimum		1.15	95.83
Maximum		4.70	108.20
Mean		2.57	100.78
StdDev		1.08	3.22
N		14	14
41	(26)		
Minimum		1.15	94.44
Maximum		4.70	108.20
Mean		2.57	100.67
StdDev		1.09	3.44
N		14	14

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
NEOMYCIN STUDIES

TIME in minutes		AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
42	(27)		
Minimum		1.12	93.33
Maximum		4.70	108.20
Mean		2.57	100.76
StdDev		1.09	3.85
N		14	14
43	(28)		
Minimum		1.10	91.67
Maximum		4.70	108.20
Mean		2.57	100.49
StdDev		1.10	4.18
N		14	14
44	(29)		
Minimum		1.10	91.67
Maximum		4.70	108.20
Mean		2.57	100.49
StdDev		1.10	4.18
N		14	14
45	(30)		
Minimum		1.10	91.67
Maximum		4.70	108.20
Mean		2.57	100.49
StdDev		1.10	4.18
N		14	14

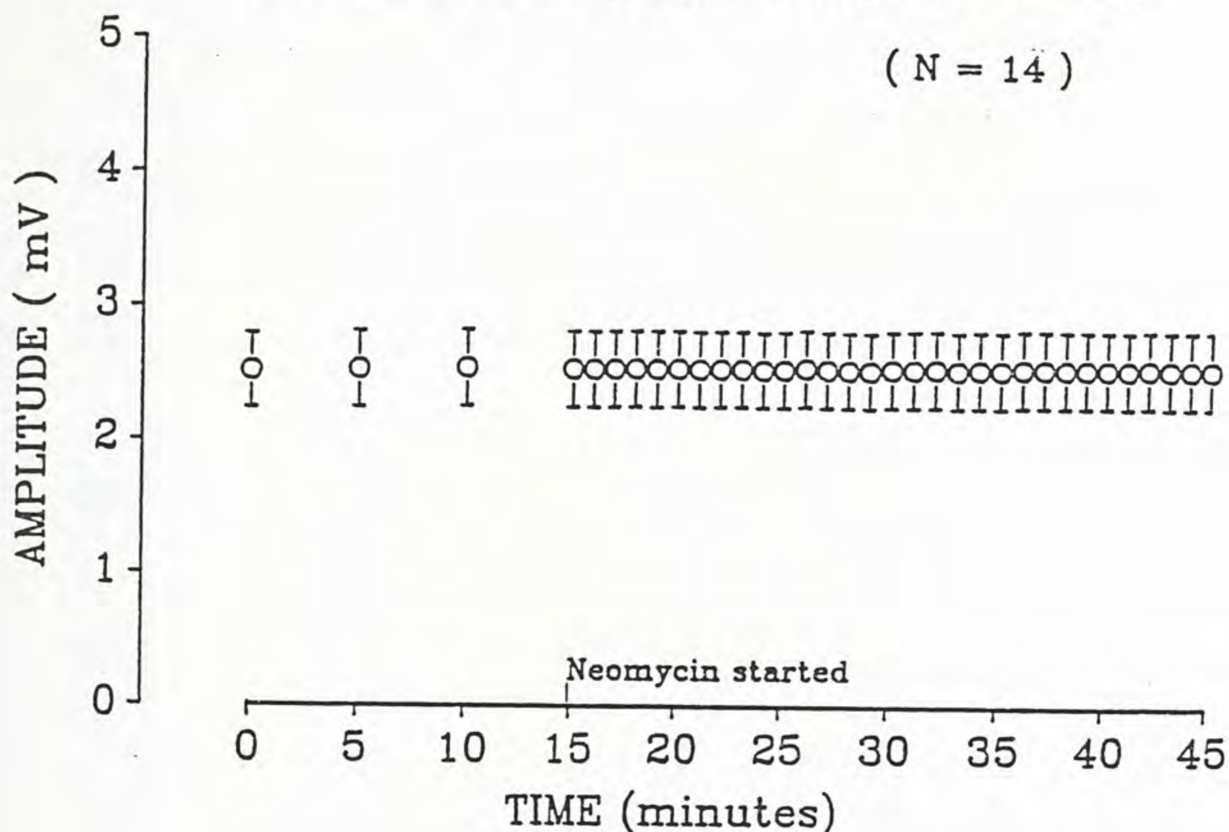


Figure 5 are the data of A compound action potentials of 14 frog sciatic nerves treated with 5.7 mM neomycin in Ringer solution for 30 minutes . Neomycin was given from 15 minutes onward. Peak amplitude was plotted against time . Results are expressed as mean $\pm$SEM. No significant change was found before and after the exposure of neomycin solution ($P \geq 0.05$) .

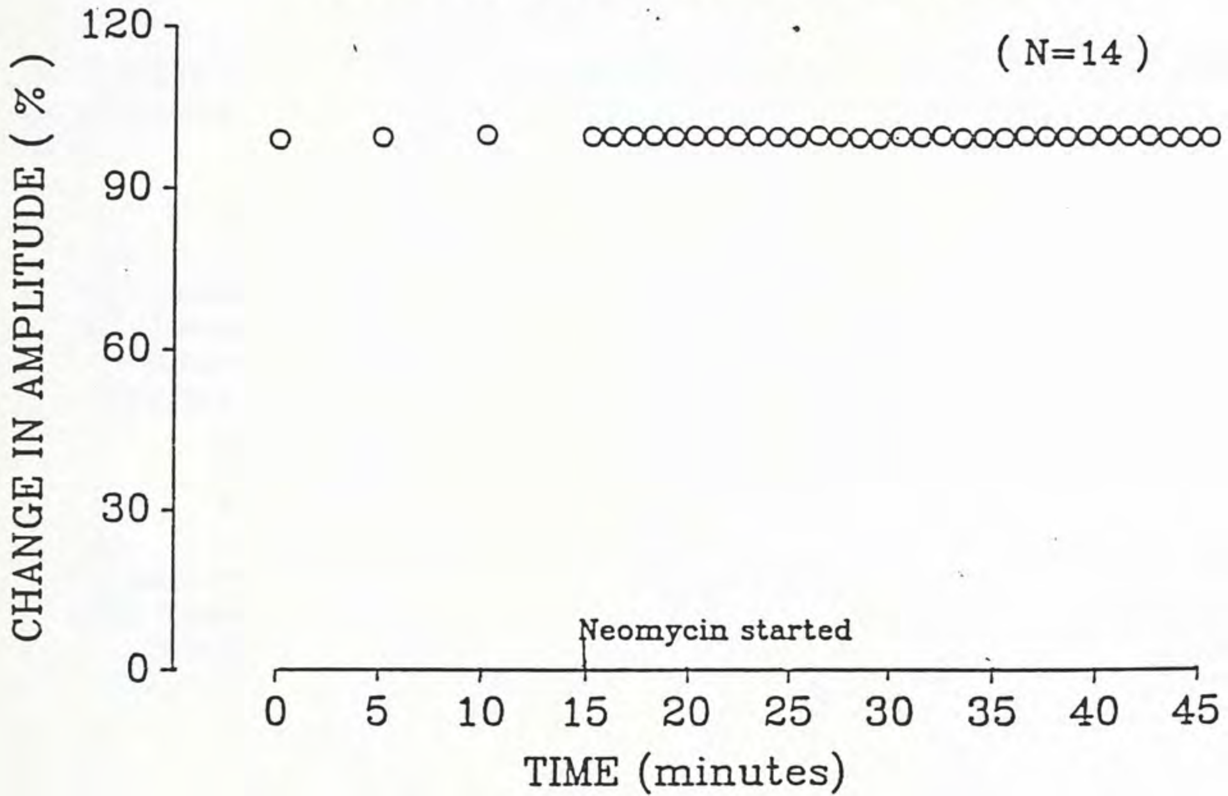


Figure 6 are the data of A compound action potentials of 14 frog sciatic nerve treated with 5.7 mM neomycin for 30 minutes in Ringer solution. Neomycin was given from 15 minute onward. Change in amplitude was plotted against the time. Results were expressed as mean $\pm$SEM. No significant change was found before and after the exposure of neomycin solution (P $\geq$0.05) .

TABLE 9

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
NEOMYCIN STUDIES

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
0		
Minimum	18.8	94.02
Maximum	25.0	100.00
Mean	21.5	99.57
StdDev	1.9	1.60
N	14	14
5		
Minimum	18.8	94.02
Maximum	25.0	100.00
Mean	21.5	99.57
StdDev	1.9	1.60
N	14	14
10		
Minimum	18.8	100.00
Maximum	25.0	100.00
Mean	21.6	100.00
StdDev	1.9	0.00
N	14	14
15 (Neomycin started)		
Minimum	18.8	100.00
Maximum	25.0	100.00
Mean	21.6	100.00
StdDev	1.9	0.00
N	14	14
16 (1)		
Minimum	18.8	90.87
Maximum	25.0	100.00
Mean	21.4	99.35
StdDev	1.9	2.44
N	14	14

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
NEOMYCIN STUDIES

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
17 (2)		
Minimum	18.8	90.87
Maximum	25.0	105.13
Mean	21.5	99.71
StdDev	1.8	2.89
N	14	14
18 (3)		
Minimum	18.8	90.87
Maximum	25.0	100.00
Mean	21.4	99.35
StdDev	1.9	2.44
N	14	14
19 (4)		
Minimum	18.8	90.87
Maximum	25.0	100.00
Mean	21.4	99.35
StdDev	1.9	2.44
N	14	14
20 (5)		
Minimum	18.8	90.87
Maximum	25.0	100.00
Mean	21.4	99.35
StdDev	1.9	2.44
N	14	14
21 (6)		
Minimum	18.8	90.87
Maximum	25.0	105.13
Mean	21.4	99.35
StdDev	1.8	3.15
N	14	14

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
NEOMYCIN STUDIES

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
22 (7)		
Minimum	18.8	94.94
Maximum	25.0	105.13
Mean	21.5	99.70
StdDev	1.8	2.30
N	14	14
23 (8)		
Minimum	17.6	93.81
Maximum	25.0	105.13
Mean	21.4	99.26
StdDev	1.9	2.78
N	14	14
24 (9)		
Minimum	17.6	93.81
Maximum	25.0	105.13
Mean	21.4	99.26
StdDev	1.9	2.78
N	14	14
25 (10)		
Minimum	17.6	93.81
Maximum	25.0	105.13
Mean	21.4	99.26
StdDev	1.9	2.78
N	14	14
26 (11)		
Minimum	18.8	94.94
Maximum	25.0	105.13
Mean	21.6	100.24
StdDev	1.7	2.18
N	14	14

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
NEOMYCIN STUDIES

TIME in minutes		CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
27	(12)		
Minimum		17.6	93.81
Maximum		25.0	105.13
Mean		21.5	99.56
StdDev		2.0	2.59
N		14	14
28	(13)		
Minimum		18.8	94.94
Maximum		25.0	105.13
Mean		21.6	100.00
StdDev		1.8	2.00
N		14	14
29	(14)		
Minimum		18.8	94.94
Maximum		25.0	105.13
Mean		21.6	100.00
StdDev		1.8	2.00
N		14	14
30	(15)		
Minimum		17.6	93.81
Maximum		25.0	105.13
Mean		21.5	99.56
StdDev		2.0	2.59
N		14	14
31	(16)		
Minimum		18.8	94.94
Maximum		25.0	106.19
Mean		21.6	100.45
StdDev		1.7	2.59
N		14	14

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
NEOMYCIN STUDIES

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
32 (17)		
Minimum	18.8	94.94
Maximum	25.0	100.00
Mean	21.4	99.36
StdDev	1.9	1.65
N	14	14
33 (18)		
Minimum	18.8	94.94
Maximum	25.0	105.13
Mean	21.6	100.00
StdDev	1.8	2.00
N	14	14
34 (19)		
Minimum	18.8	94.94
Maximum	25.0	105.13
Mean	21.6	100.00
StdDev	1.8	2.00
N	14	14
35 (20)		
Minimum	18.8	94.94
Maximum	25.0	105.13
Mean	21.6	100.00
StdDev	1.8	2.00
N	14	14
36 (21)		
Minimum	18.8	94.39
Maximum	25.0	105.13
Mean	21.5	99.60
StdDev	1.9	2.50
N	14	14

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
NEOMYCIN STUDIES

TIME in minutes		CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
37	(22)		
Minimum		18.8	94.94
Maximum		25.0	105.13
Mean		21.6	100.00
StdDev		1.8	2.00
N		14	14
38	(23)		
Minimum		18.8	94.94
Maximum		25.0	105.13
Mean		21.6	100.00
StdDev		1.8	2.00
N		14	14
39	(24)		
Minimum		18.8	94.94
Maximum		25.0	100.00
Mean		21.5	99.64
StdDev		1.8	1.35
N		14	14
40	(25)		
Minimum		18.8	94.94
Maximum		25.0	100.00
Mean		21.5	99.64
StdDev		1.8	1.35
N		14	14
41	(26)		
Minimum		18.8	94.94
Maximum		25.0	100.00
Mean		21.5	99.64
StdDev		1.8	1.35
N		14	14

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
NEOMYCIN STUDIES

TIME in minutes		CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
42	(27)		
Minimum		18.8	94.94
Maximum		25.0	100.00
Mean		21.5	99.64
StdDev		1.8	1.35
N		14	14
43	(28)		
Minimum		18.8	94.94
Maximum		25.0	100.00
Mean		21.5	99.64
StdDev		1.8	1.35
N		14	14
44	(29)		
Minimum		18.8	94.94
Maximum		25.0	100.00
Mean		21.5	99.64
StdDev		1.8	1.35
N		14	14
45	(30)		
Minimum		18.8	94.94
Maximum		25.0	103.24
Mean		21.5	99.87
StdDev		1.8	1.66
N		14	14

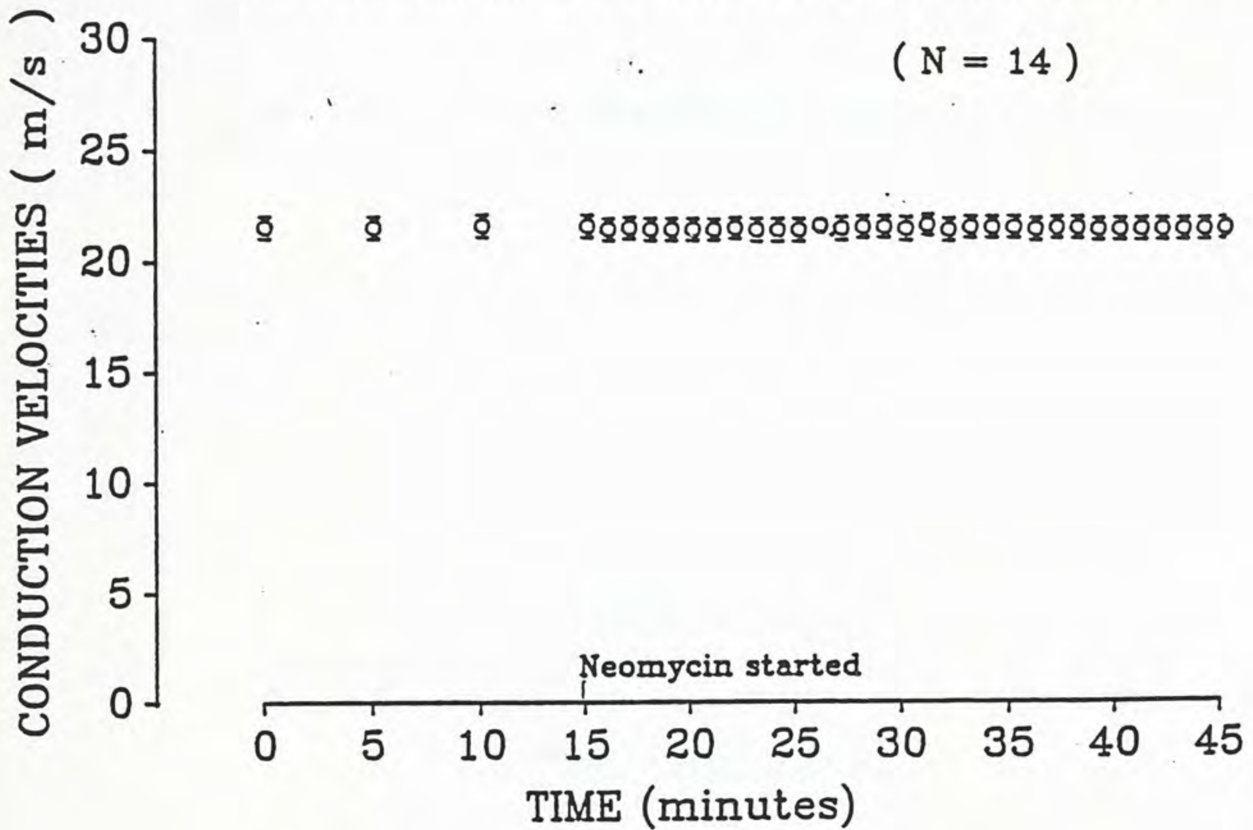


Figure 7 are the data of A compound action potentials of 14 frog sciatic nerves treated with 5.7 mM neomycin in Ringer solution for 30 minutes. Neomycin was given from 15 minutes onward. Conduction velocity was plotted against the time. Results were expresssed as mean $\pm$SEM. No significant difference was found before and after the exposure to neomycin solution ($P > 0.05$).

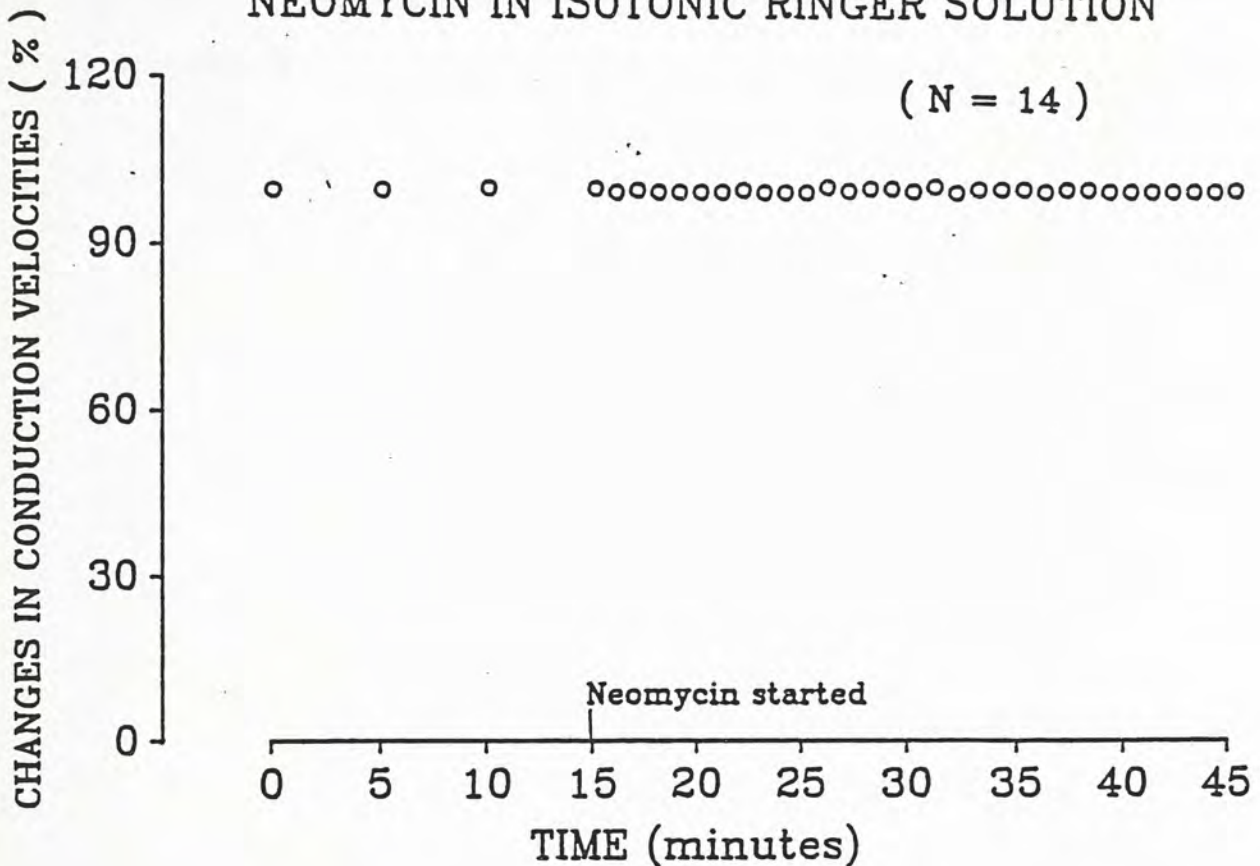


Figure 8 are the data of A compound action potentials of 14 frog sciatic nerves treated with 5.7 mM neomycin in Ringer solution for 30 minutes. Neomycin was given from 15 minutes onward. change in Conduction velocity was plotted against the time. Results were expressed as mean ± SEM. No significant difference was found before and after exposure to neomycin solution ($P > 0.05$).

The digitized recording of the effect of acute exposure of neomycin on the nervous conduction is shown in figure 9.

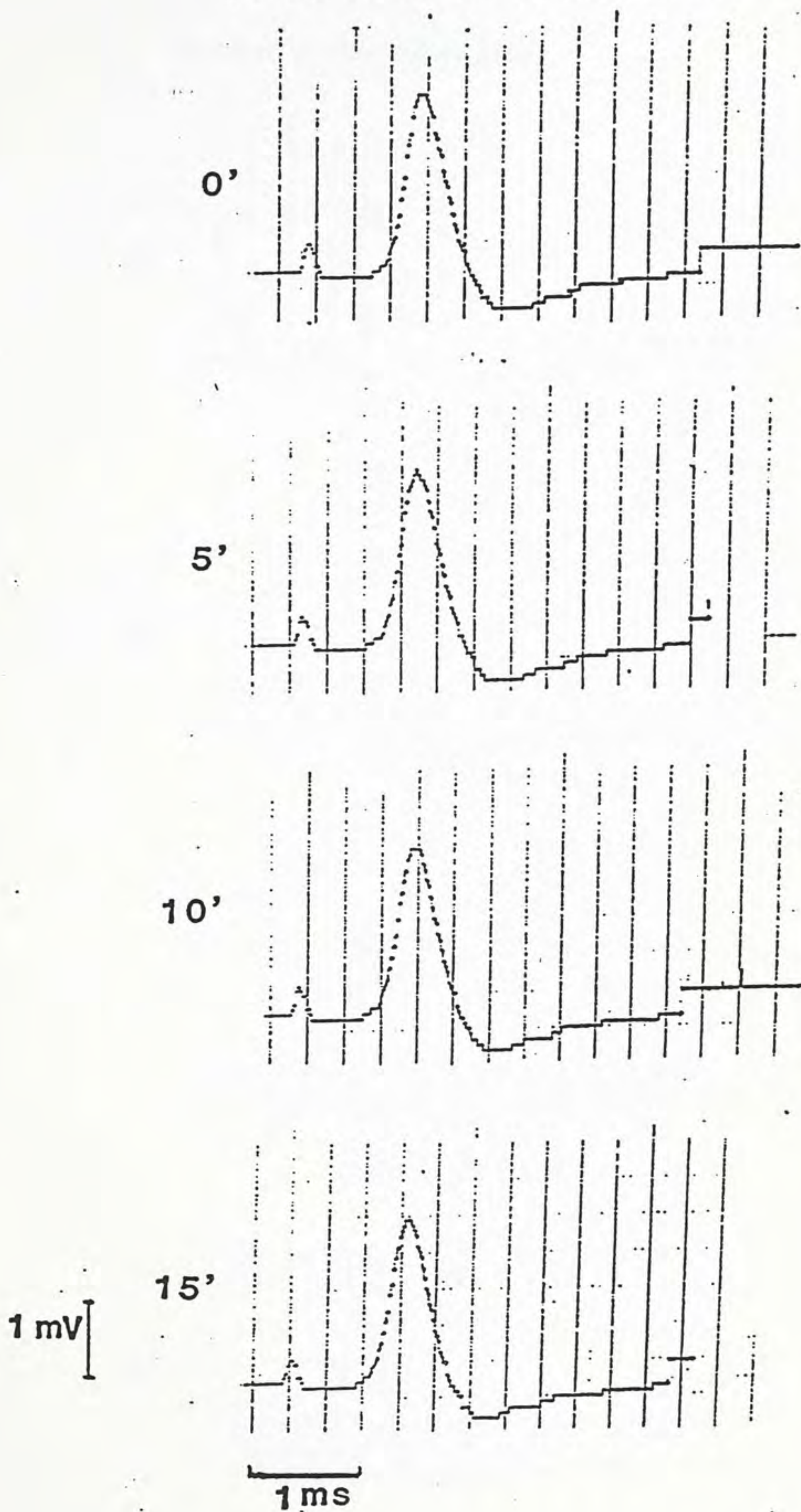
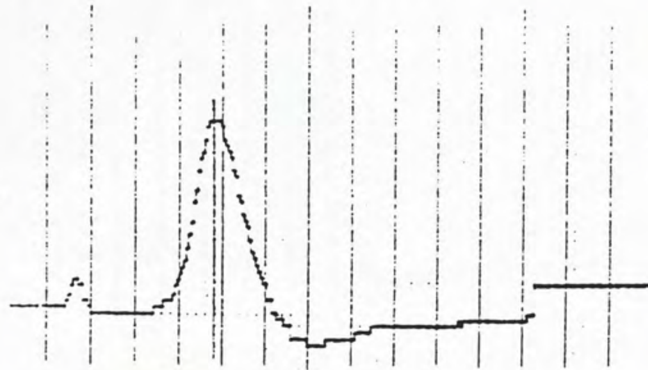


Figure 9 In vitro study of action potential in frog sciatic nerve, original recording of the action potential before and during exposure tn 5.7 mM neomycin solution in Ringer solution for 30 minutes. Conductive distance was 3 cm.

Neomycin started

1'



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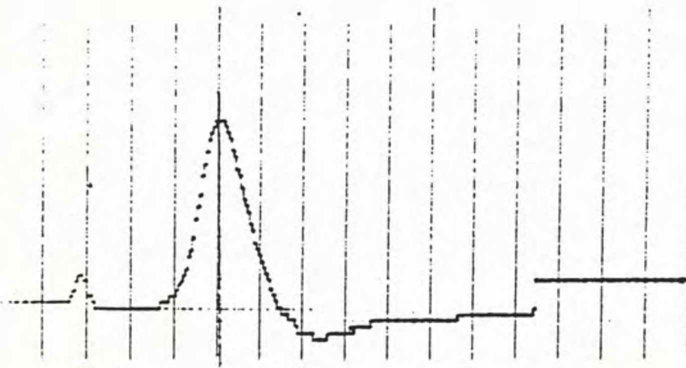
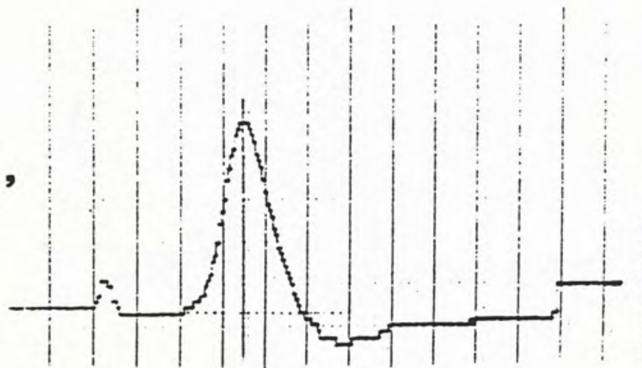


3'



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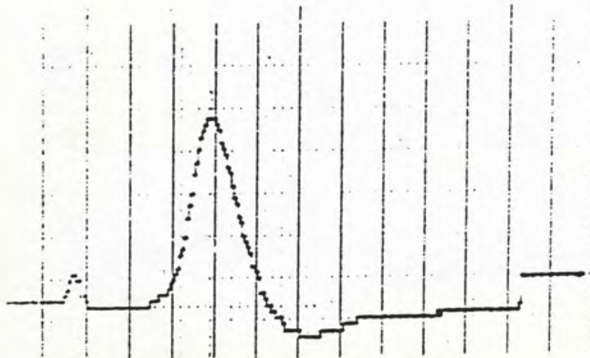

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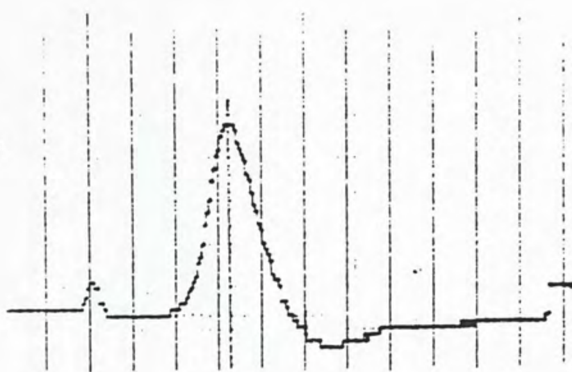
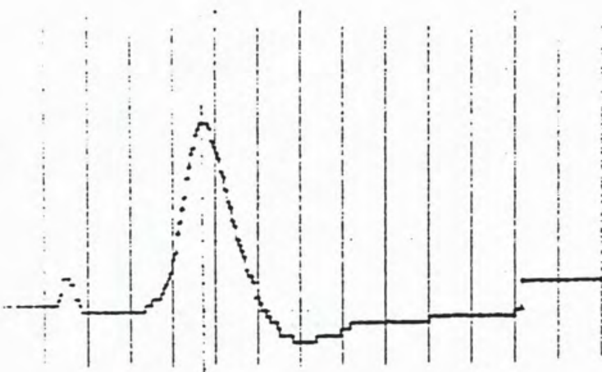


6’



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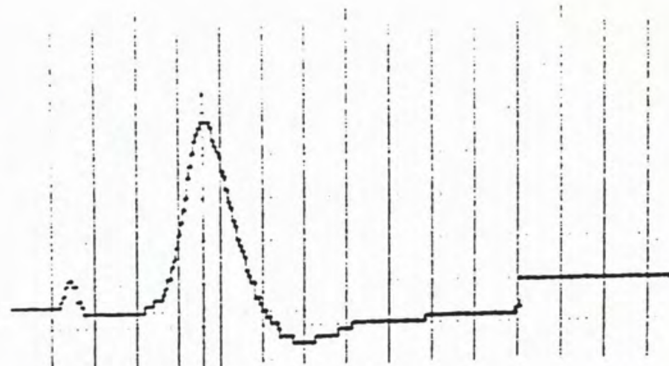

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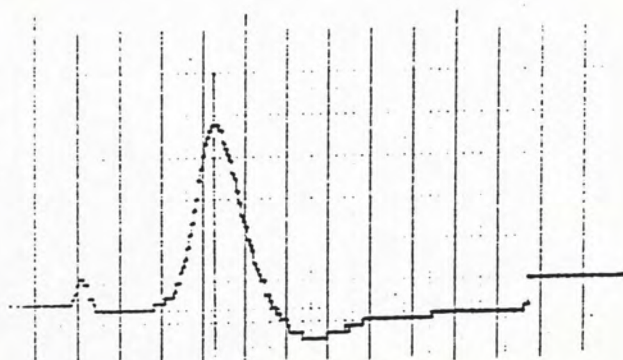

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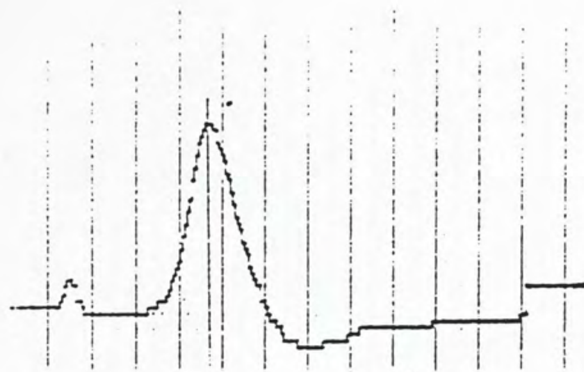

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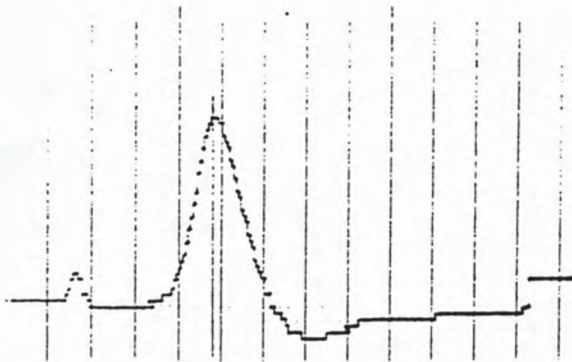

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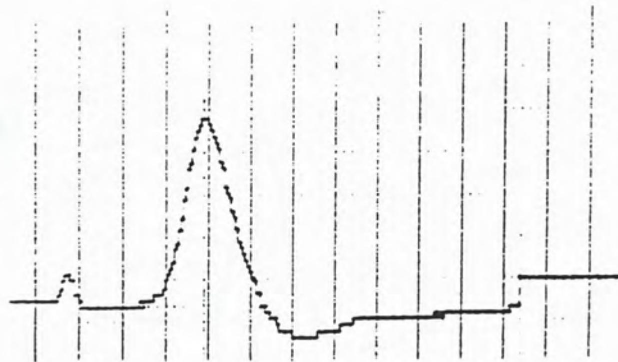

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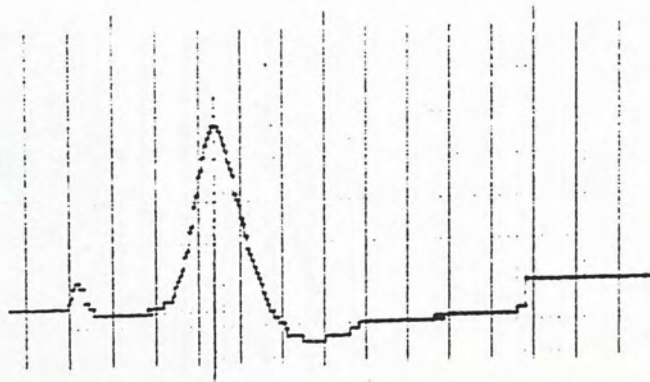
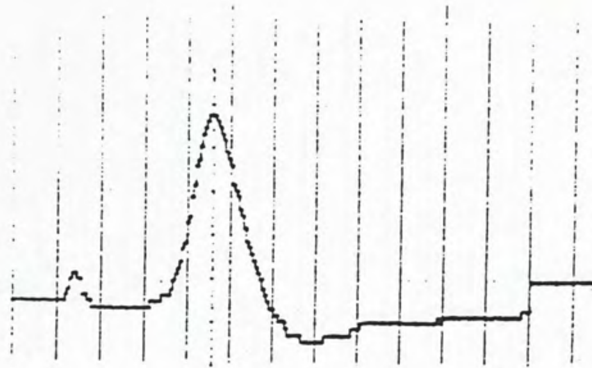


13'



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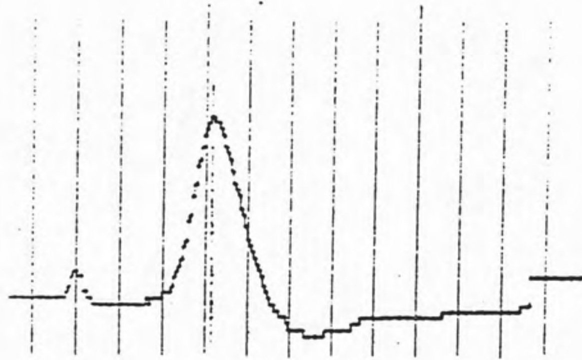

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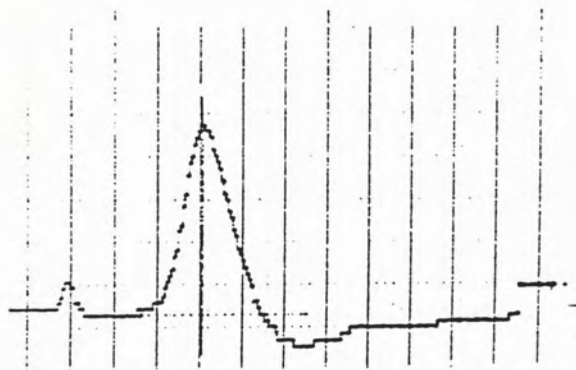
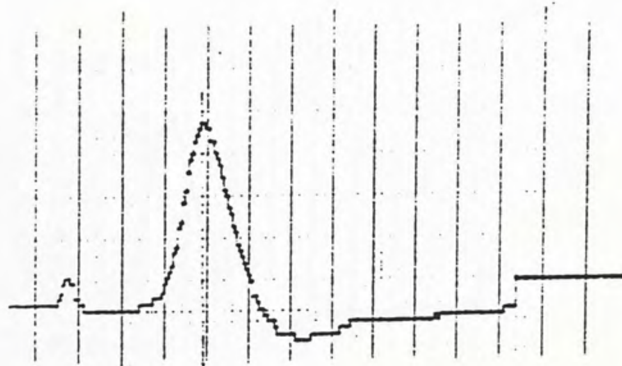
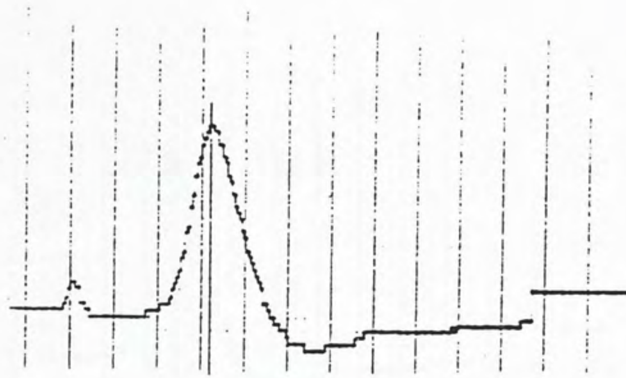


16'



17’



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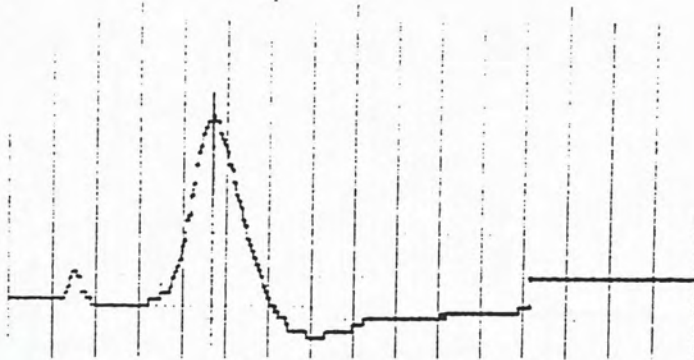
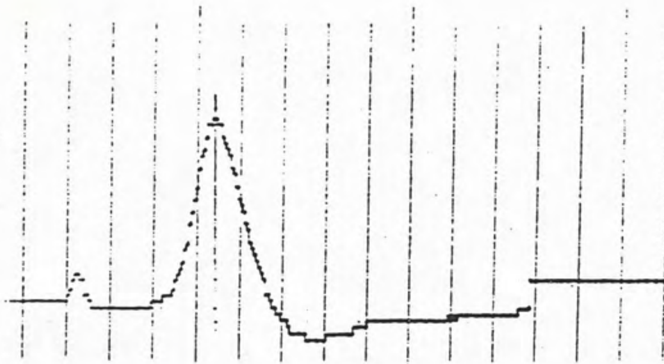


19’



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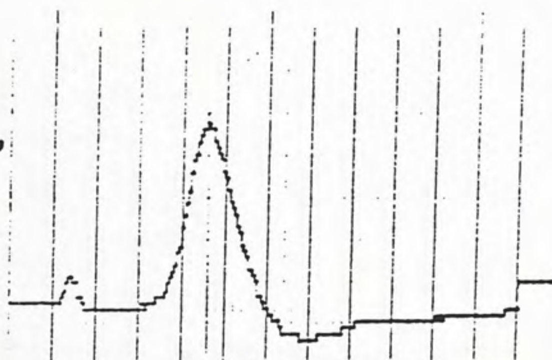
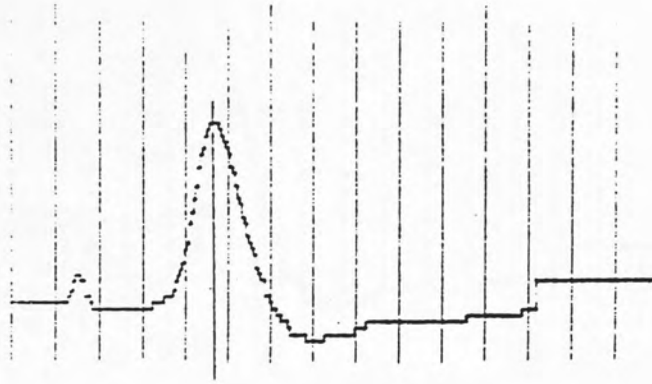


21’



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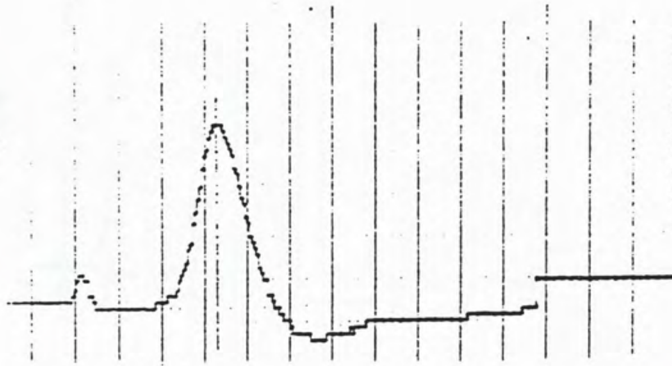

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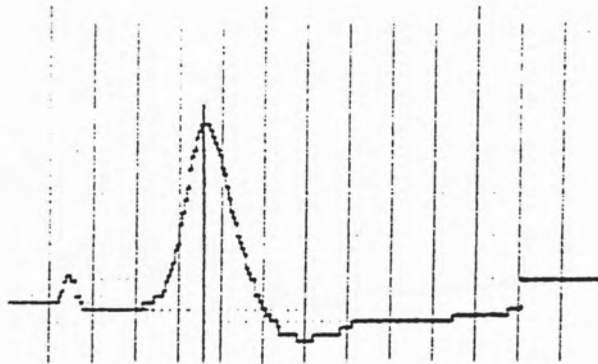
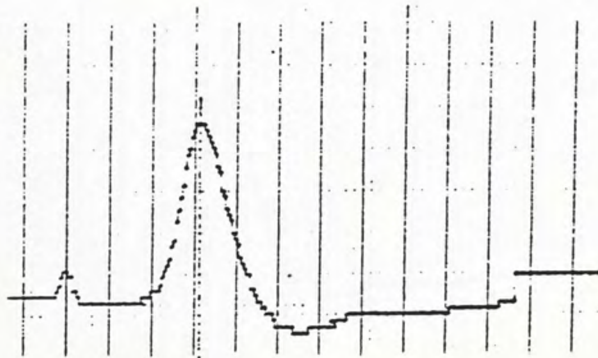


24’



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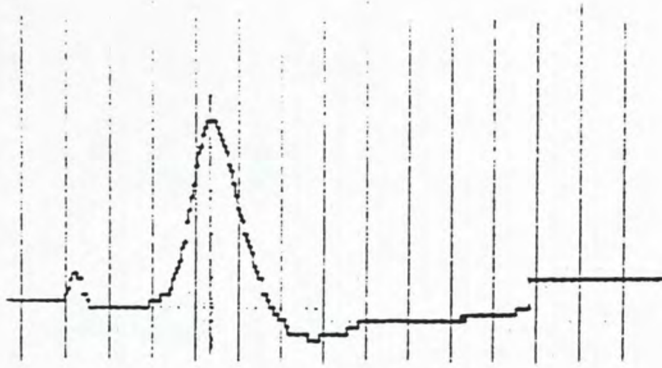
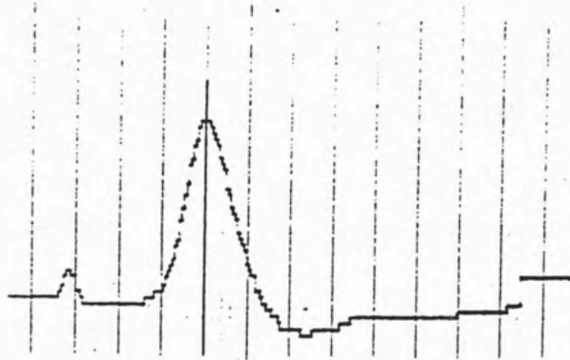
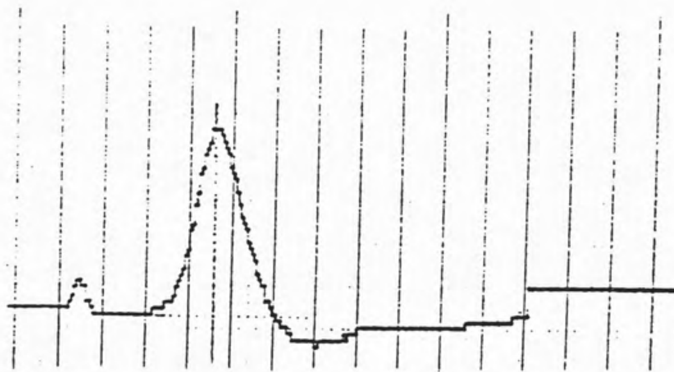


26'



27'



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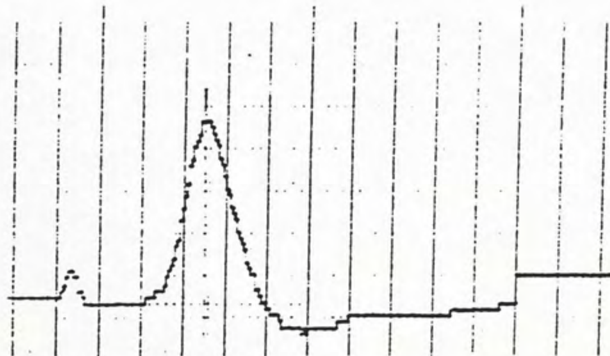
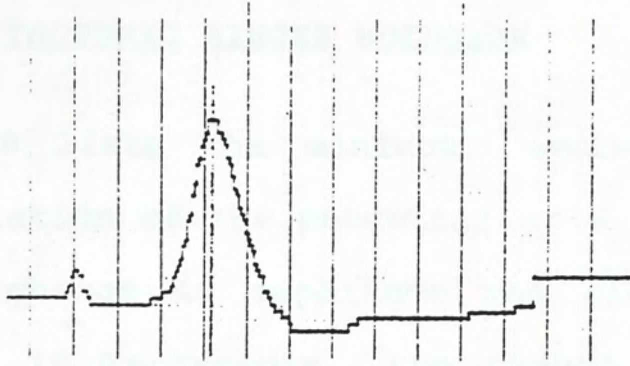
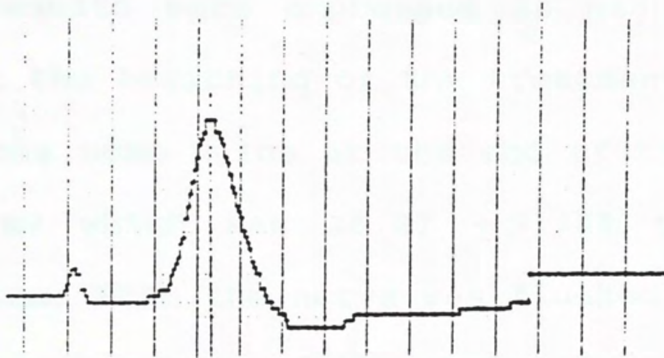


29'



30'



20 mM KCL IN ISOTONIC RINGER SOLUTION

Table 10 lists the minimum, maximum, mean and standard deviation of the potential of 6 frogs sciatic nerves. The change in amplitude was plotted against time. Figure 10 represents the change in amplitude with time. Results were expressed as mean ± SEM. The mean value at the beginning of the treatment was 1.77 ± 0.16 mV and the mean value at the end of treatment was 0.50 ± 0.19 mV which was 26.87 ± 9.98% of the pre-treatment value. When the nerve was flushed with Ringer solution, gradual return of the amplitude was shown. By 15 minutes, the mean value was 1.55 ±0.09 mv which was 96.94 ±1.8 % of the pre-treatment value). Significant different between change in amplitude with time was found at 14 and 15 minutes after the application of KCL solution.($p < 0.05$). When the value obtained from the first stimulus was taken as 100%, the change in amplitude with time is shown in figure 11.

CONDUCTION VELOCITIES

Table 11 lists the minimum, maximum, mean and standard deviation of the conduction velocities of 6 frogs sciatic nerves. The change in conduction velocity was plotted against time. Figure 12 represents the change in conduction velocity with time. Results were expressed as mean ±SEM. The mean value at the beginning of the treatment was 23.6 ±0.9 m/s and the mean value

at the end of treatment was 14.7 $\pm$0.8 m/s. When the nerve was flushed with Ringer solution, there was gradual increase of the conduction velocity. At 15 minutes, the mean value was 22.0 $\pm$1.9 m/s. When the value obtained before the application of KCL solution was taken as 100%, the change in conduction velocity with time is shown in figure 13.

Figure 14 shows the sequence of blockade of the action potential with the exposure of 20 mM KCL in isotonic Ringer solution.

TABLE 10

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
20 mM KCL IN ISOTONIC RINGER SOLUTION

TIME in minutes	AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
0		
Minimum	1.34	95.83
Maximum	2.30	103.08
Mean	1.76	99.82
StdDev	.35	2.31
N	6	6
5		
Minimum	1.34	96.67
Maximum	2.40	103.08
Mean	1.77	99.96
StdDev	.39	2.03
N	6	6
10		
Minimum	1.34	98.00
Maximum	2.40	103.08
Mean	1.77	101.18
StdDev	.39	1.63
N	6	6
15 (20 mM KCL in isotonic Ringer solution started)		
Minimum	1.30	100.00
Maximum	2.40	100.00
Mean	1.77	100.00
StdDev	.39	0.00
N	6	6
16 (1)		
Minimum	.94	62.67
Maximum	2.05	100.00
Mean	1.50	83.97
StdDev	.44	12.50
N	6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
20 mM KCL IN ISOTONIC RINGER SOLUTION

TIME in minutes		AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
17	(2)		
Minimum		.81	54.00
Maximum		2.00	95.56
Mean		1.37	76.33
StdDev		.49	16.64
N		6	6
18	(3)		
Minimum		.55	34.38
Maximum		1.90	96.11
Mean		1.23	67.68
StdDev		.61	25.70
N		6	6
19	(4)		
Minimum		.31	20.67
Maximum		1.83	101.67
Mean		1.15	62.84
StdDev		.69	32.03
N		6	6
20	(5)		
Minimum		.25	16.67
Maximum		1.66	92.22
Mean		1.02	55.45
StdDev		.66	30.91
N		6	6
21	(6)		
Minimum		.19	12.67
Maximum		1.65	91.67
Mean		.96	52.05
StdDev		.63	30.19
N		6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
20 mM KCL IN ISOTONIC RINGER SOLUTION

TIME in minutes	AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
22 (7)		
Minimum	.19	12.67
Maximum	1.58	87.78
Mean	.90	49.08
StdDev	.60	30.18
N	6	6
23 (8)		
Minimum	.19	12.67
Maximum	1.51	83.89
Mean	.82	44.45
StdDev	.57	28.31
N	6	6
24 (9)		
Minimum	.18	11.25
Maximum	1.51	83.89
Mean	.76	41.54
StdDev	.57	28.79
N	6	6
25 (10)		
Minimum	.18	11.25
Maximum	1.44	80.00
Mean	.73	39.30
StdDev	.55	27.98
N	6	6
26 (11)		
Minimum	.18	11.25
Maximum	1.37	76.11
Mean	.66	36.05
StdDev	.52	26.70
N	6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
20 mM KCL IN ISOTONIC RINGER SOLUTION

TIME in minutes		AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
27	(12)		
Minimum		.18	11.25
Maximum		1.30	72.22
Mean		.64	34.71
StdDev		.51	25.88
N		6	6
28	(13)		
Minimum		.12	7.50
Maximum		1.30	72.22
Mean		.59	32.04
StdDev		.51	26.37
N		6	6
29	(14)		
Minimum		.09	5.62
Maximum		1.22	67.78
Mean		.53	29.05
StdDev		.49	25.68
N		6	6
30	(15) "wash with Ringer solution"		
Minimum		.06	4.00
Maximum		1.15	63.89
Mean		.50	26.87
StdDev		.47	24.45
N		6	6
31	"1"		
Minimum		.09	6.21
Maximum		1.51	83.89
Mean		.69	40.90
StdDev		.54	29.24
N		6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
20 mM KCL IN ISOTONIC RINGER SOLUTION

TIME in minutes	AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
32 "2"		
Minimum	.21	14.48
Maximum	1.68	93.33
Mean	.89	52.83
StdDev	.60	32.12
N	6	6
33 "3"		
Minimum	.26	18.57
Maximum	1.74	96.67
Mean	1.15	70.55
StdDev	.50	27.47
N	6	6
34 "4"		
Minimum	.26	18.57
Maximum	1.80	100.00
Mean	1.21	73.87
StdDev	.52	28.92
N	6	6
35 "5"		
Minimum	.57	40.71
Maximum	1.80	100.00
Mean	1.30	79.85
StdDev	.43	20.74
N	6	6
36 "6"		
Minimum	.88	62.86
Maximum	1.80	100.00
Mean	1.36	84.44
StdDev	.32	13.00
N	6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
20 mM KCL IN ISOTONIC RINGER SOLUTION

TIME in minutes		AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
37	"7"		
Minimum		.98	70.00
Maximum		1.83	101.67
Mean		1.41	87.83
StdDev		.30	11.33
N		6	6
38	"8"		
Minimum		1.08	77.14
Maximum		1.83	101.67
Mean		1.45	90.21
StdDev		.28	8.71
N		6	6
39	"9"		
Minimum		1.19	85.00
Maximum		1.83	101.67
Mean		1.47	91.52
StdDev		.26	6.72
N		6	6
40	"10"		
Minimum		1.24	88.50
Maximum		1.83	101.67
Mean		1.51	93.89
StdDev		.25	5.95
N		6	6
41	"11"		
Minimum		1.24	88.50
Maximum		1.83	101.67
Mean		1.52	94.48
StdDev		.25	5.58
N		6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF AMPLITUDE
20 mM KCL IN ISOTONIC RINGER SOLUTION

TIME in minutes		AMPLITUDE in mV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
42	"12"		
Minimum		1.30	88.50
Maximum		1.83	101.67
Mean		1.53	95.54
StdDev		.23	4.80
N		6	6
43	"13"		
Minimum		1.30	90.50
Maximum		1.83	101.67
Mean		1.54	96.23
StdDev		.23	4.19
N		6	6
44	"14"		
Minimum		1.30	90.50
Maximum		1.83	101.67
Mean		1.55	96.94
StdDev		.23	4.44
N		6	6
45	"15"		
Minimum		1.30	90.50
Maximum		1.83	101.67
Mean		1.55	96.94
StdDev		.23	4.44
N		6	6

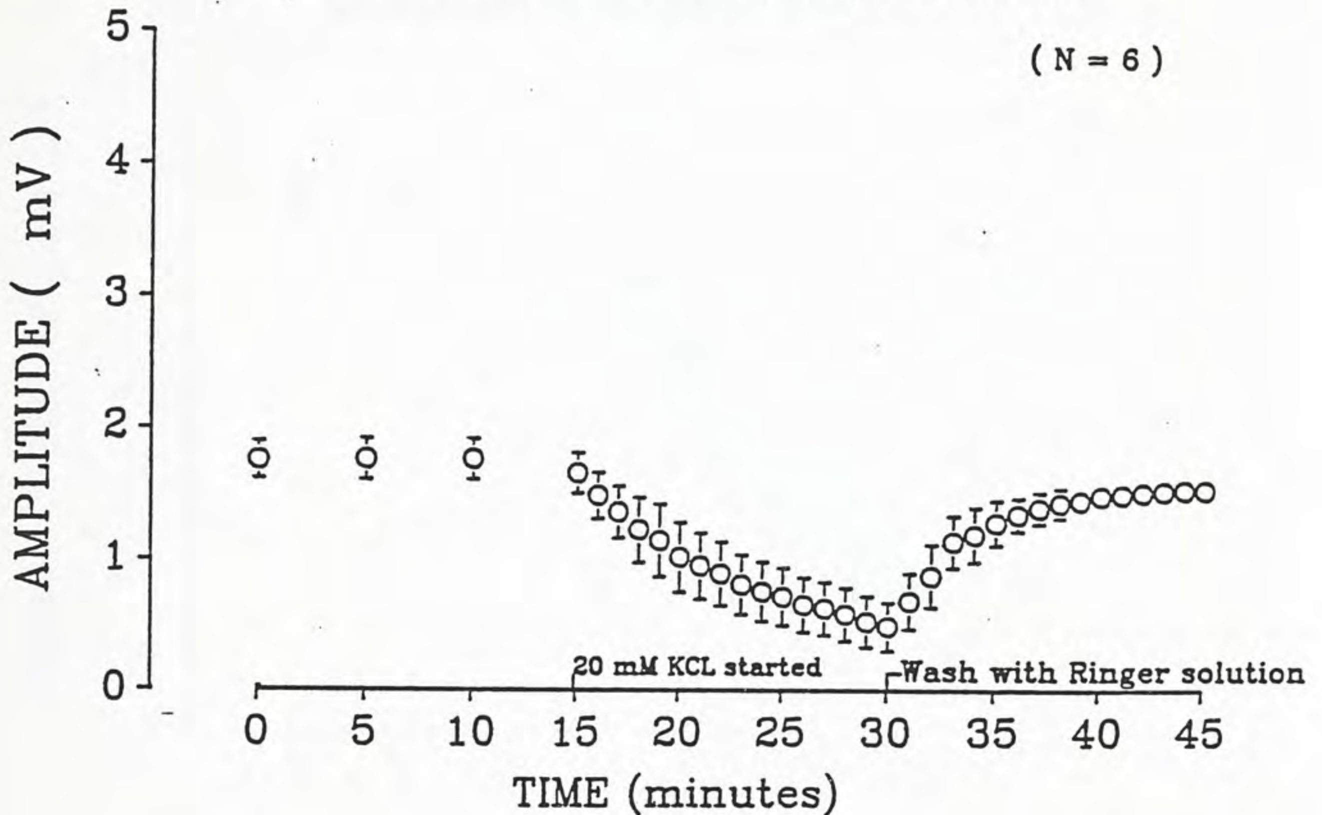


Figure 10 are the data of A compound action potentials of 6 frog sciatic nerves treated with 20 mM KCL in isotonic Ringer solution for 15 minutes and was subsequently washed with Ringer solution for another 15 minutes . Peak amplitude was plotted against the time . Results were expressed as mean $\pm$ SEM. Significant difference from control values was found at 14 and 15 minutes after application of 20 mM KCL in isotonic Ringer solution ($P \leq 0.05$).

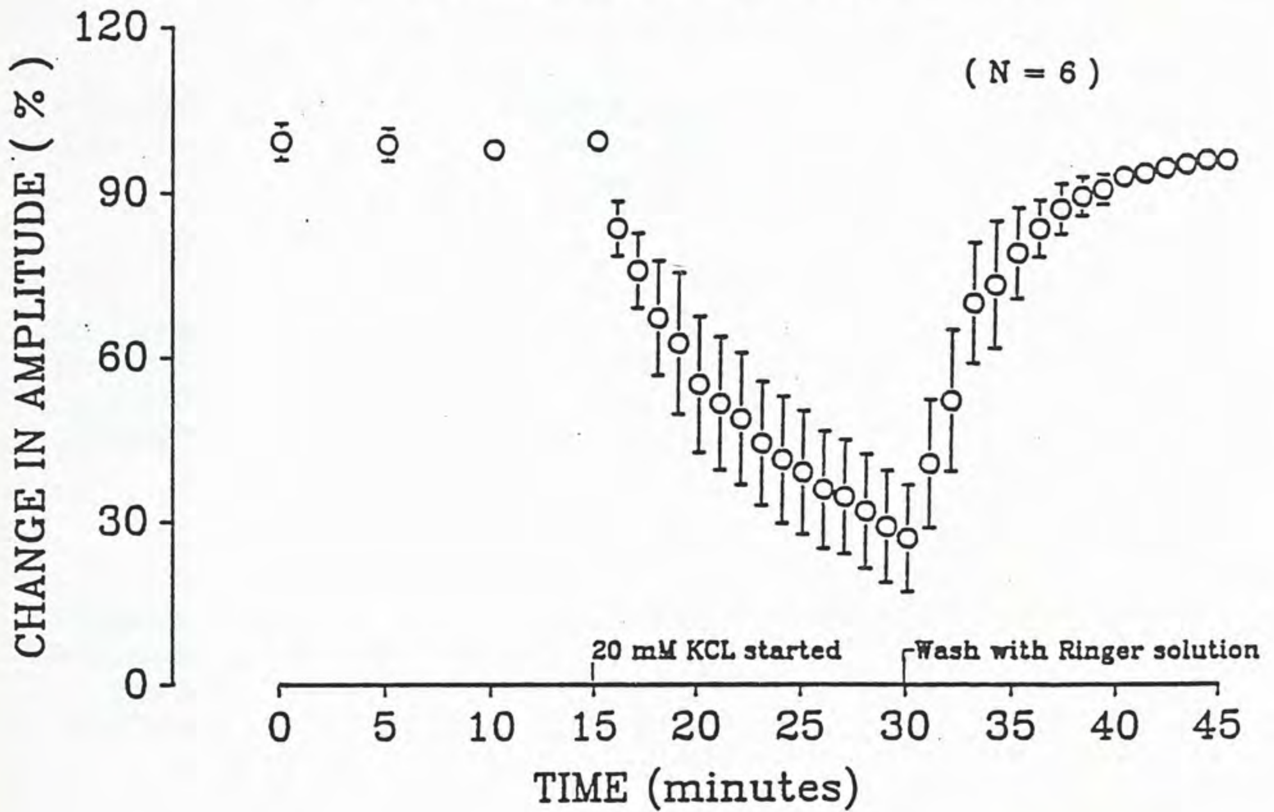


Figure 11 are the data of A compound action potentials of 6 frog sciatic nerves treated with 20 mM KCL in isotonic Ringer solution for 15 minutes and was subsequently washed with Ringer solution. The amplitude before the application of KCL was taken as 100%. Change in amplitude was plotted against the time. Results were expressed as mean $\pm$ SEM. Significant difference from control values was found at 14 and 15 minutes after application of 20 mM KCL in isotonic Ringer solution ($p \leq 0.05$).

TABLE 11

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
20 mM KCL IN ISOTONIC RINGER SOLUTION

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
0		
Minimum	20.0	88.68
Maximum	27.7	110.97
Mean	23.5	99.41
StdDev	2.9	8.27
N	6	6
5		
Minimum	20.0	88.68
Maximum	27.7	110.97
Mean	23.4	99.00
StdDev	2.6	7.41
N	6	6
10		
Minimum	19.0	94.33
Maximum	25.0	100.00
Mean	23.2	98.24
StdDev	2.3	2.73
N	6	6
15 (20 mM KCL in isotonic Ringer solution started)		
Minimum	20.0	100.00
Maximum	25.0	100.00
Mean	23.6	100.00
StdDev	1.9	0.00
N	6	6
16 (1)		
Minimum	14.4	72.41
Maximum	25.0	100.00
Mean	20.5	86.49
StdDev	3.4	9.25
N	6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
20 mM KCL IN ISOTONIC RINGER SOLUTION

TIME in minutes	CONDUCTION VELOCITY in m/s	— CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
17 (2)		
Minimum	13.4	67.36
Maximum	22.7	90.87
Mean	19.1	80.69
StdDev	3.4	10.66
N	6	6
18 (3)		
Minimum	11.3	56.86
Maximum	22.7	90.84
Mean	18.3	76.65
StdDev	4.1	13.18
N	6	6
19 (4)		
Minimum	12.0	60.00
Maximum	22.1	88.68
Mean	18.2	76.46
StdDev	3.7	11.41
N	6	6
20 (5)		
Minimum	15.5	69.01
Maximum	21.6	86.57
Mean	18.6	78.73
StdDev	2.4	7.31
N	6	6
21 (6)		
Minimum	15.4	64.38
Maximum	21.1	84.50
Mean	17.9	75.66
StdDev	2.4	7.49
N	6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
20 mM KCL IN ISOTONIC RINGER SOLUTION

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
22 (7)		
Minimum	14.4	61.29
Maximum	22.0	95.74
Mean	18.0	76.43
StdDev	3.5	13.59
N	6	6
23 (8)		
Minimum	14.4	60.00
Maximum	20.1	80.51
Mean	16.9	71.65
StdDev	2.5	7.91
N	6	6
24 (9)		
Minimum	13.6	57.89
Maximum	20.6	82.48
Mean	16.5	69.68
StdDev	3.0	9.81
N	6	6
25 (10)		
Minimum	13.5	56.35
Maximum	19.9	79.86
Mean	16.2	68.35
StdDev	2.7	8.78
N	6	6
26 (11)		
Minimum	13.5	56.35
Maximum	19.9	79.86
Mean	16.0	67.61
StdDev	2.7	9.17
N	6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
20 mM KCL IN ISOTONIC RINGER SOLUTION

TIME in minutes		CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
27	(12)		
Minimum		13.5	56.35
Maximum		19.0	76.06
Mean		15.6	66.13
StdDev		2.4	8.05
N		6	6
28	(13)		
Minimum		13.4	53.85
Maximum		18.2	73.01
Mean		15.4	65.27
StdDev		2.0	7.15
N		6	6
29	(14)		
Minimum		13.4	53.85
Maximum		18.2	73.01
Mean		15.1	64.27
StdDev		2.1	7.79
N		6	6
30	(15)	"Wash with Ringer solution"	
Minimum		12.6	53.85
Maximum		18.2	73.01
Mean		14.7	62.24
StdDev		2.1	6.84
N		6	6
31	"1"		
Minimum		10.2	42.86
Maximum		18.9	72.76
Mean		14.3	56.31
StdDev		3.3	10.67
N		6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
20 mM KCL IN ISOTONIC RINGER SOLUTION

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
32 "2"		
Minimum	12.3	51.42
Maximum	18.9	72.76
Mean	15.9	62.80
StdDev	2.4	7.26
N	6	6
33 "3"		
Minimum	13.4	55.84
Maximum	19.7	72.76
Mean	16.9	66.53
StdDev	2.4	6.71
N	6	6
34 "4"		
Minimum	15.4	64.13
Maximum	20.7	79.93
Mean	17.9	70.51
StdDev	2.3	6.40
N	6	6
35 "5"		
Minimum	14.9	55.17
Maximum	21.8	83.75
Mean	17.9	70.74
StdDev	3.1	10.39
N	6	6
36 "6"		
Minimum	15.4	61.68
Maximum	21.7	83.75
Mean	18.3	72.44
StdDev	2.4	8.42
N	6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
20 mM KCL IN ISOTONIC RINGER SOLUTION

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
37 "7"		
Minimum	16.5	66.67
Maximum	21.9	84.40
Mean	19.2	75.81
StdDev	2.1	7.05
N	6	6
38 "8"		
Minimum	16.5	69.01
Maximum	21.9	84.40
Mean	19.6	77.47
StdDev	2.2	5.96
N	6	6
39 "9"		
Minimum	16.5	69.01
Maximum	21.9	84.40
Mean	19.7	77.92
StdDev	2.1	5.95
N	6	6
40 "10"		
Minimum	18.0	75.18
Maximum	24.1	88.89
Mean	20.7	81.66
StdDev	2.1	5.10
N	6	6
41 "11"		
Minimum	18.9	80.00
Maximum	24.1	88.89
Mean	21.3	84.15
StdDev	1.8	3.19
N	6	6

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
20 mM KCL IN ISOTONIC RINGER SOLUTION

TIME in minutes		CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
42	"12"		
Minimum		18.9	82.51
Maximum		24.1	90.84
Mean		21.7	85.47
StdDev		1.9	3.53
N		6	6
43	"13"		
Minimum		18.9	82.51
Maximum		24.1	90.84
Mean		21.7	85.47
StdDev		1.9	3.53
N		6	6
44	"14"		
Minimum		19.8	82.51
Maximum		24.1	90.84
Mean		22.0	86.95
StdDev		1.9	3.13
N		6	6
45	"15"		
Minimum		19.8	82.51
Maximum		24.1	90.84
Mean		22.0	86.95
StdDev		1.9	3.13
N		6	6

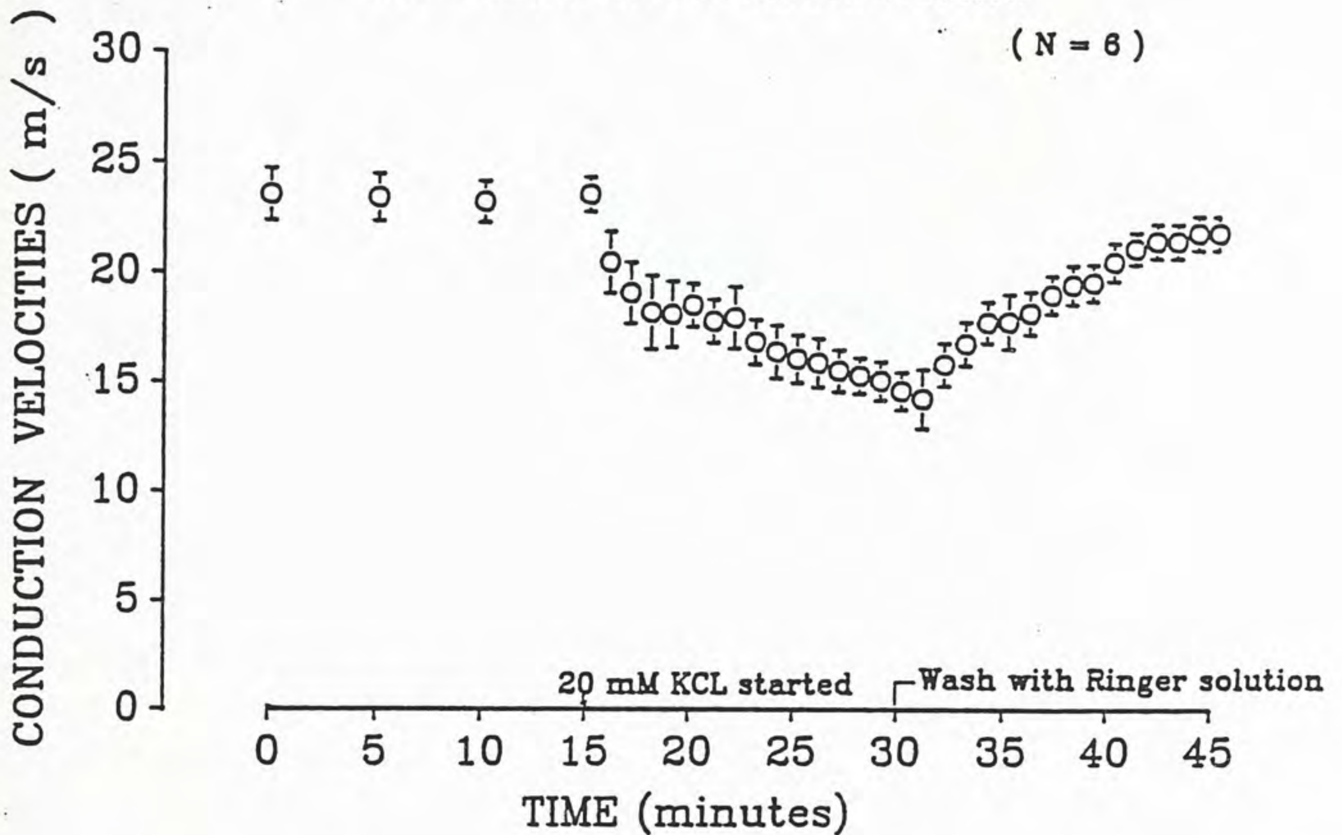


Figure 12 are the data of A compound action potentials of 6 frog sciatic nerve treated with 20 mM KCL in isotonic Ringer solution for 15 minutes and was subsequently washed with Ringer solution for 15 minutes. Conduction velocity was plotted against the time. Results were expressed as mean ± SEM. Significant difference from contorl values was found at 14 and 15 minutes after the application of KCL solution ($P \leq 0.05$).

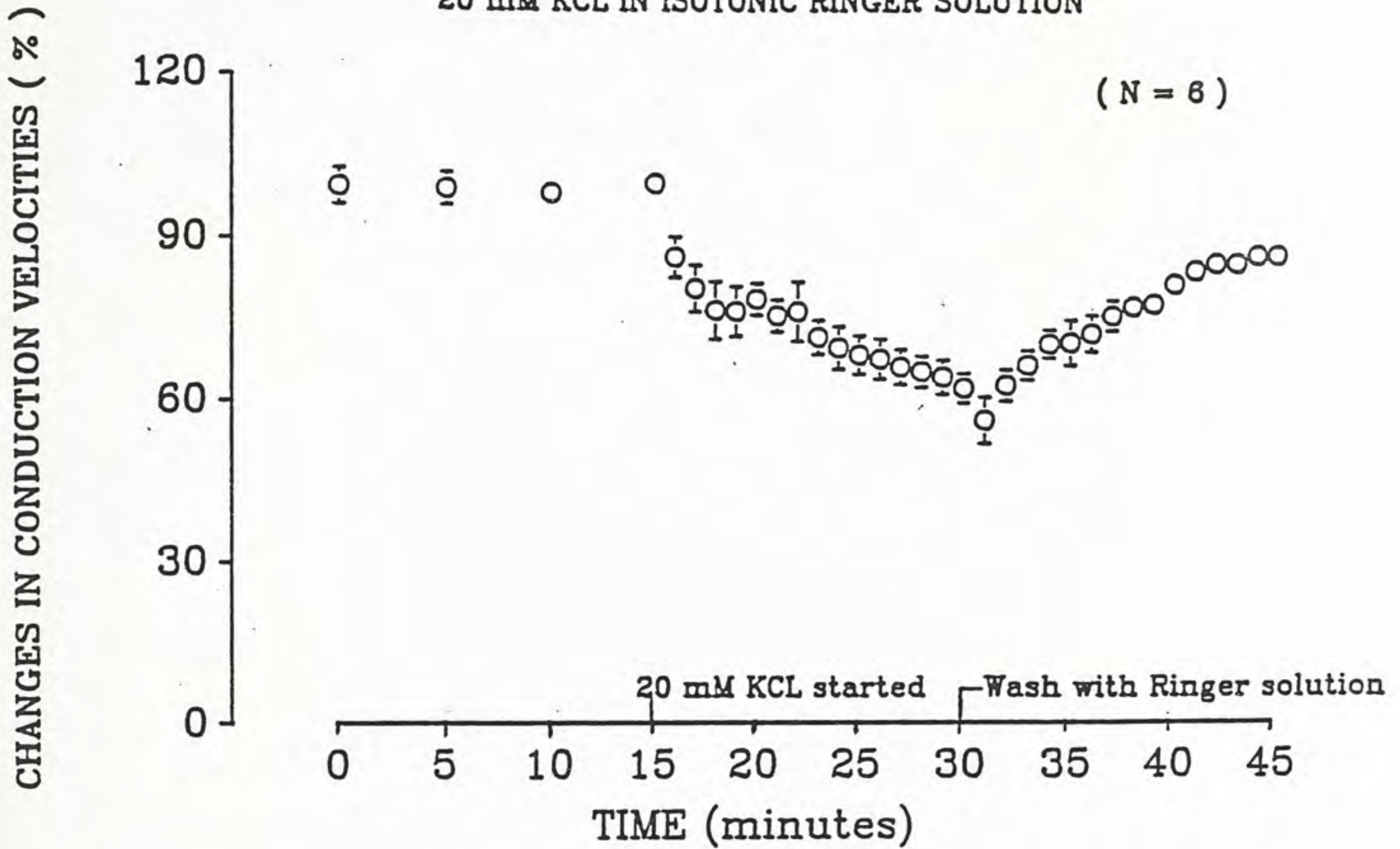


Figure 13 are the data of A compound action potentials of 6 frog sciatic nerves treated with 20 mM KCL in isotonic Ringer solution for 15 minutes and was subsequently washed with Ringer solution. The conduction velocity before the application of KCL was taken as 100 %. Change in conduction velocity was plotted against the time. Results were expressed as mean ± SEM. Significant difference from contorl values was found at 14 and 15 minutes after the application of KCL solution ($P \leq 0.05$).

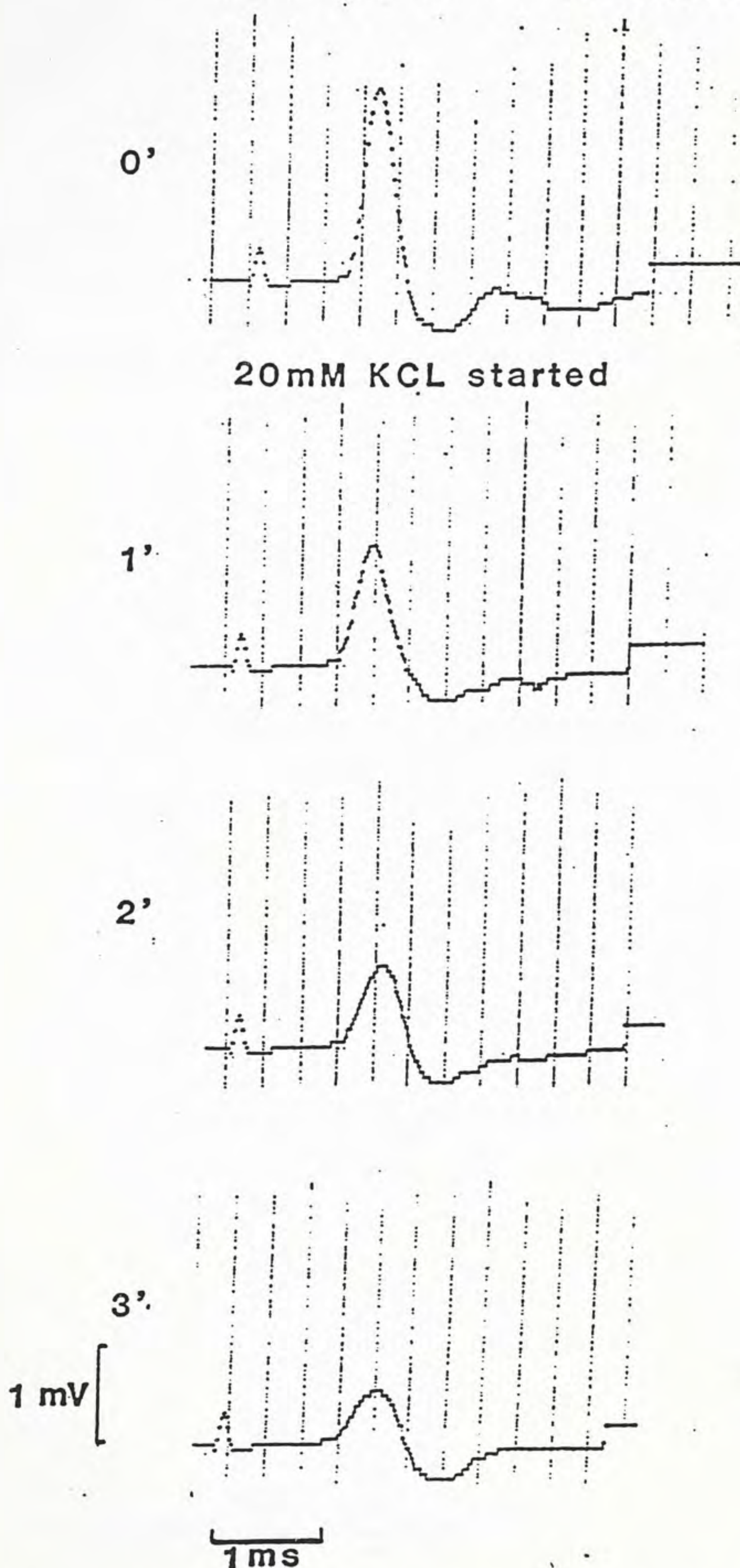
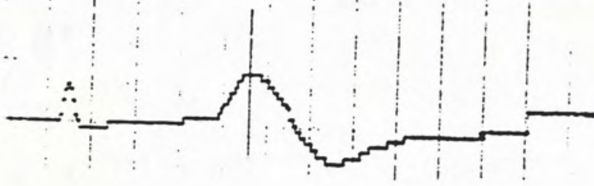


Figure 14 In vitro study of action potential in frog sciatic nerve, original recording of the action potential with exposure to 20 mM KCL in isotonic Ringer solution for 15 minutes. The nerve was subsequently washed with Ringer solution to show the gradual return of the action potential. Conductive distance was 3 cm.

4'



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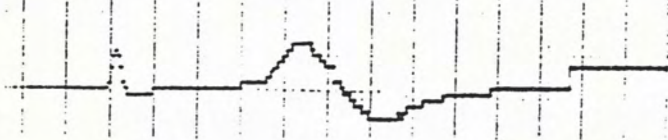
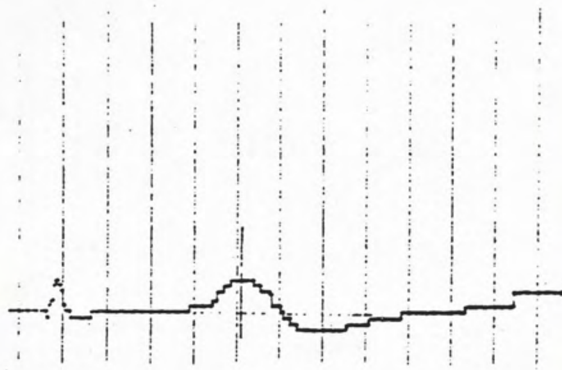
6'



7'

8'



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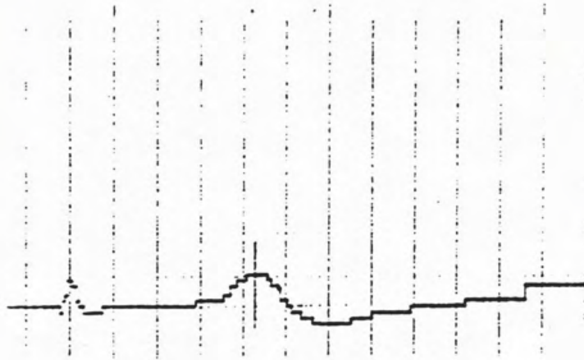

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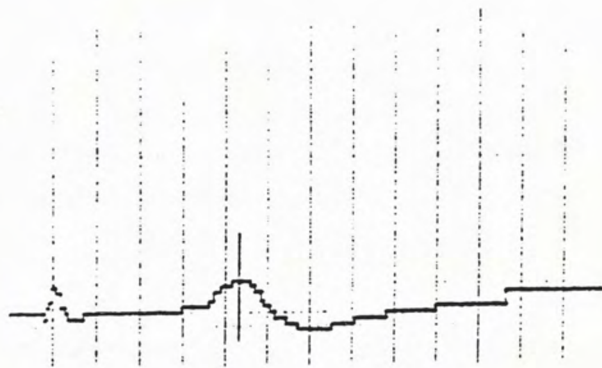

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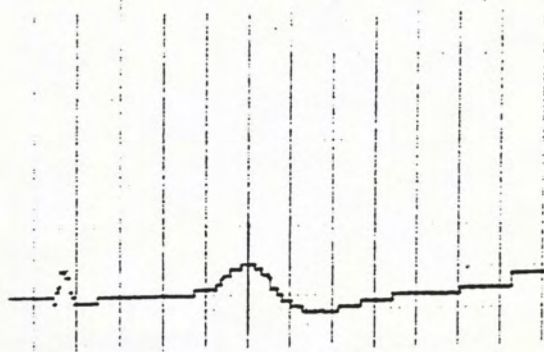

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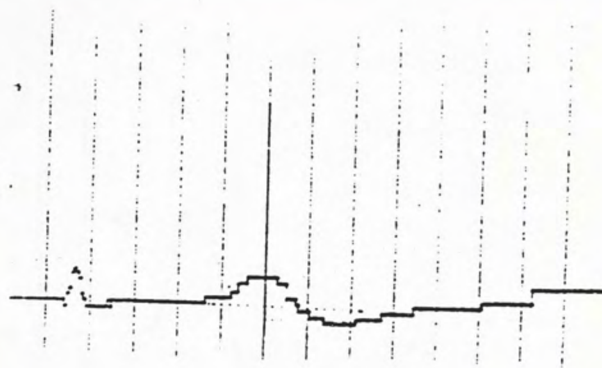

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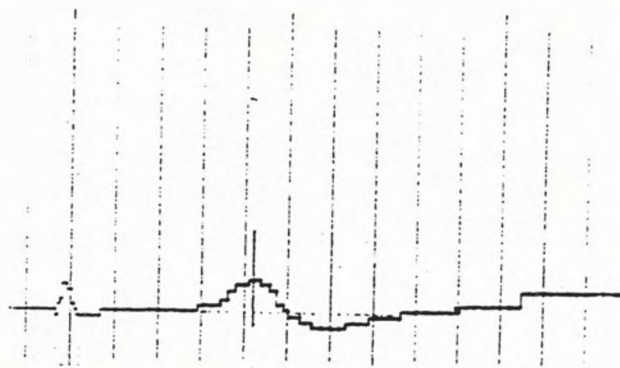

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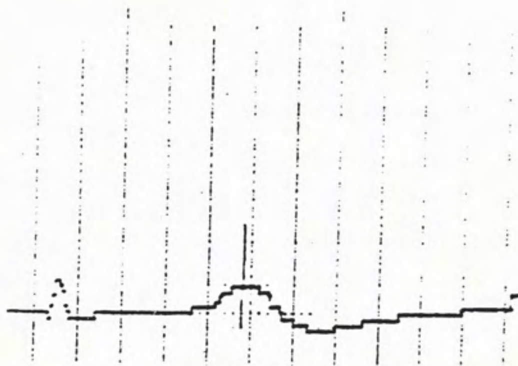

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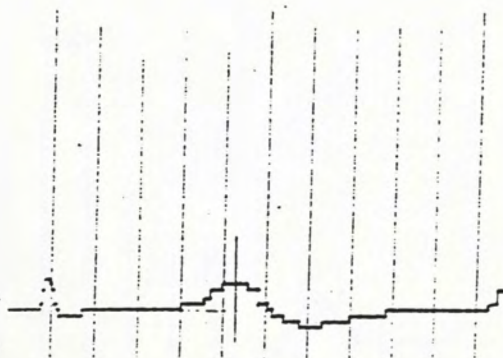
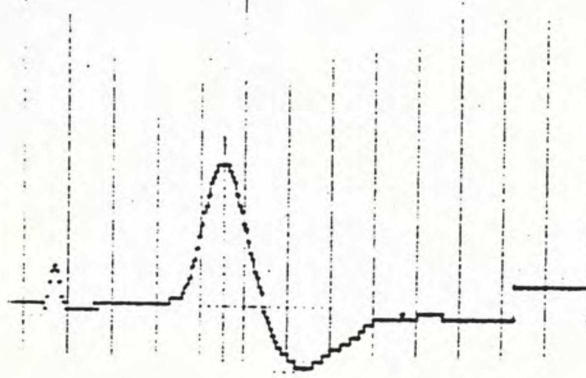


Wash with Ringer solution

1'



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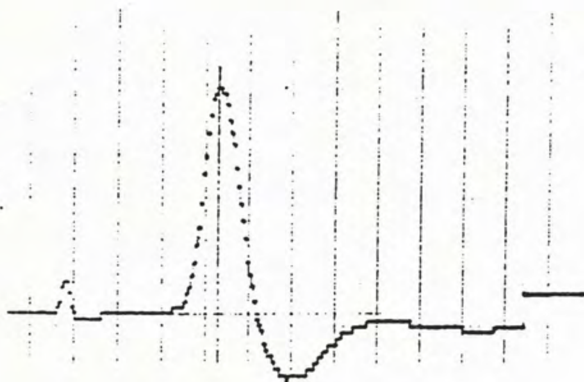

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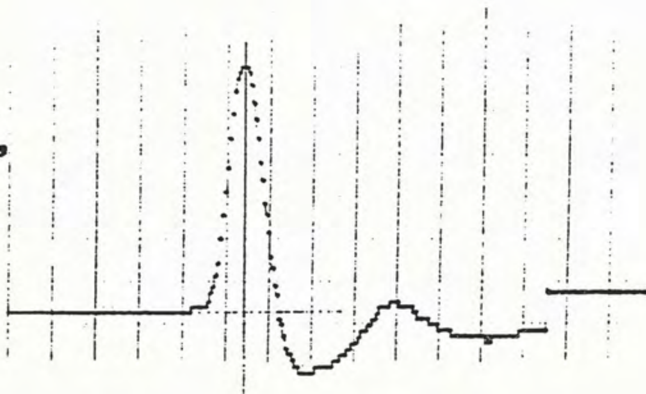

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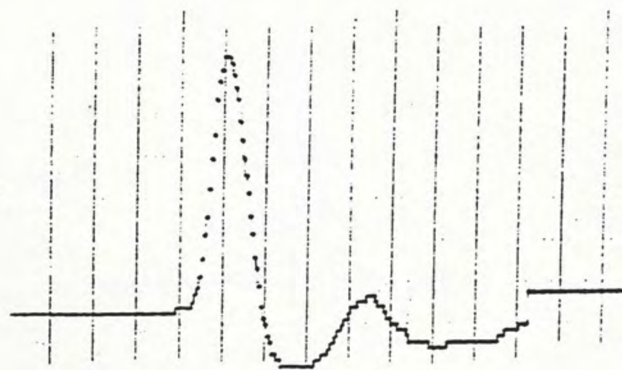

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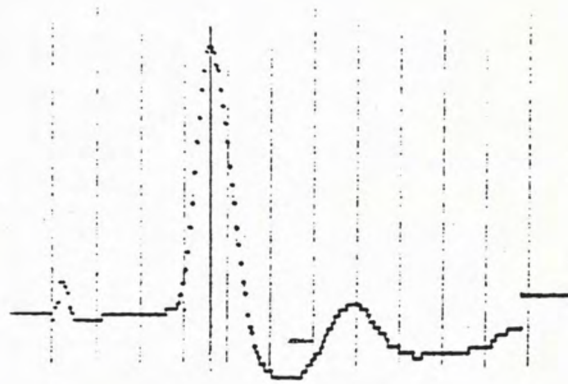

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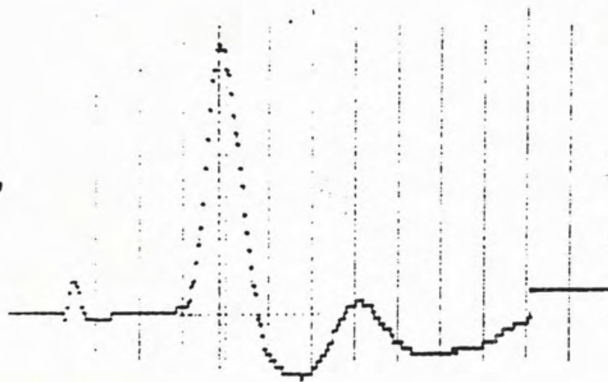

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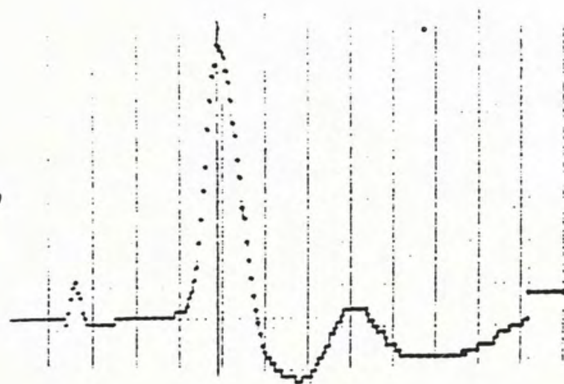

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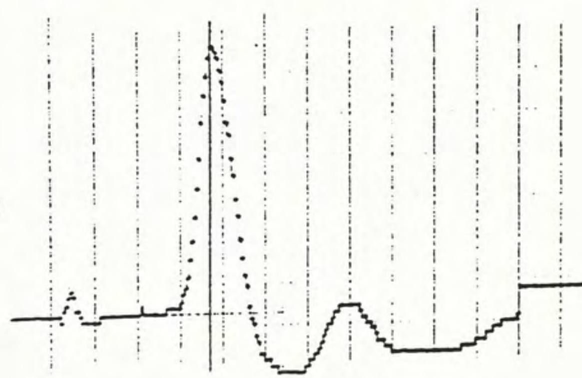

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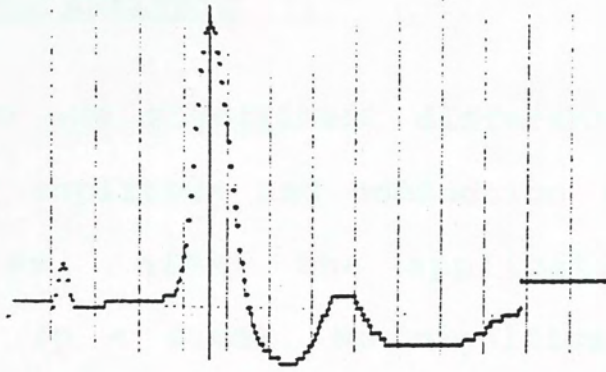

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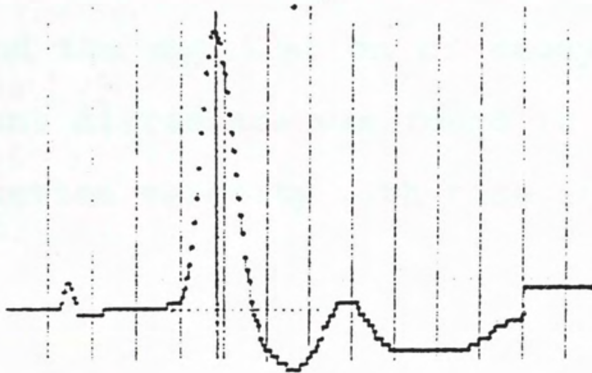


STATISTICAL ANALYSIS

There was significant difference from control in change of amplitude and conduction velocity at 14 and 15 minutes after the application of potassium chloride. ($p < 0.05$). No significant difference was found in change of amplitude and conduction velocity before and the application of neomycin ($p > 0.05$). No significant difference was found in change of amplitude and conduction velocity with time in the control group.

2.2 RAT VENTROLATERAL TAIL NERVE

A COMPOUND ACTION POTENTIAL:-

Table 12 lists the minimum, maximum, mean and standard deviation of the potential from ventrolateral tail nerves of 6 rats. Stimulation was given every 5 minutes. The potential was monitored for at least 15 minutes before the middle chamber was infused with neomycin solution. The change in amplitude was plotted against the time. Figure 15 represents the change in amplitude with time. Results were expressed as mean $\pm$ SEM. The mean value before the application of neomycin solution was 20.73 $\pm$ 1.25 uV and at the end of the treatment, the mean value was 22.12 $\pm$2.98 uV. There was no significant difference between the change in amplitude with time ($p > 0.05$). When the value obtained before the start of the neomycin treatment was taken as 100%, the change in amplitude with time is shown in figure 16.

CONDUCTION VELOCITIES

Table 13 lists the minimum, maximum, mean and standard deviation of the conduction velocities from ventrolateral tail nerves of 6 rats. The change in conduction velocity was plotted against time. Figure 17 represents the change in conduction velocity with time. Results were expressed as mean $\pm$ SEM. The mean

value at the beginning of the treatment was 24.7 $\pm$0.8 m/s and at the end of the treatment, the mean value was 23.5 $\pm$ 0.8 m/s. There was no significant difference between the change in conduction velocity with time ($p > 0.05$). When the value obtained before the start of the neomycin treatment was taken as 100%, the change in conduction velocity with time is shown in figure 18.

10 mM POTASSIUM SULPHATE IN ISOTONIC KREBS SOLUTION

AMPLITUDE OF A POTENTIAL

Table 12 lists the minimum, maximum, mean and standard deviation of the potential from ventrolateral tail nerve of 6 rats. Stimulation was given every 5 minutes for one hour. The change in amplitude was plotted against time. Figure 19 represents the change in amplitude with time. Results were expressed as mean $\pm$SEM. The mean value at the beginning of the treatment was 23.94 $\pm$2.97 uV. There was mark decreased in the amplitude with respect to time upon exposure to 10 mM K_2SO_4 Krebs solution. Complete blockade of A potential was found from 5 minutes onward. When the rat-tail was flushed with Krebs solution, there was gradual return of the A potential. At 10 minutes,the mean value was 20.42 $\pm$2.43 uv. Significant difference was found at 5 and 10 minutes after the exposure to 10 mM K_2SO_4 Krebs solution ($p < 0.05$). When the value obtained before the start of potassium sulphate infusion was taken as 100%, the change in amplitude with time is shown in figure 20.

CONDUCTION VELOCITIES

Table 13 lists the minimum, maximum, mean and standard deviation of the conduction velocities from ventrolateral tail nerve of 6 rats. The change in conduction velocity was plotted against time. Figure 21

represents the change in conduction with time. Results were expressed as mean ± SEM. The mean value at the beginning of the treatment was 24.37 ± 1.84 m/s. Complete block of the potential was found from 5 minutes after exposure to 10 mM K_2SO_4 onward. When the rat-tail was flushed with Krebs solution, conduction velocity gradually increase. At 10 minutes, the mean conduction velocities was 21.94 ± 2.01 m/s. Which was 89.13 ± 8.37% of the original value. Significant difference between the change in conduction velocities with found at 5 and 10 minutes after exposure to 10 mM K_2SO_4 solution ($p < 0.05$). When the value obtained before the start of the potassium sulphate infusion was taken as 100%, the change in conduction velocity is shown in figure 22.

The original recording of the A compound action potential on the effect of neomycin and 10 mM K_2SO_4 in Krebs solution is shown in figure 23.

TABLE 12

A COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF AMPLITUDE

TIME in minutes	AMPLITUDE in μv.	CHANGE OF AMPLITUDE IN (%) OF CONTROL
0		
Minimum	13.85	61.09
Maximum	36.69	147.11
Mean	21.12	100.78
StdDev	8.19	27.74
N	6	6
5		
Minimum	16.42	72.56
Maximum	35.87	143.83
Mean	22.09	105.51
StdDev	7.43	23.44
N	6	6
10		
Minimum	16.27	79.45
Maximum	24.54	102.29
Mean	19.76	95.47
StdDev	3.32	8.48
N	6	6
15 (Neomycin started)		
Minimum	17.53	100.00
Maximum	24.94	100.00
Mean	20.73	100.00
StdDev	3.09	0.00
N	6	6
20 (5)		
Minimum	14.91	79.18
Maximum	24.93	110.16
Mean	19.46	93.59
StdDev	4.03	11.21
N	6	6

A COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF AMPLITUDE

TIME in minutes	AMPLITUDE in µv.	CHANGE OF AMPLITUDE IN (%) OF CONTROL
25 (10)		
Minimum	15.97	84.86
Maximum	23.10	108.32
Mean	19.78	95.55
StdDev	3.22	8.83
N	6	6
30 (15)		
Minimum	13.04	74.39
Maximum	26.25	119.18
Mean	20.22	97.23
StdDev	4.78	17.25
N	6	6
35 (20)		
Minimum	14.85	84.71
Maximum	27.29	122.61
Mean	20.62	99.41
StdDev	4.75	17.71
N	6	6
40 (25)		
Minimum	14.93	85.13
Maximum	27.15	133.18
Mean	21.13	102.01
StdDev	4.91	20.13
N	6	6
45 (30)		
Minimum	14.50	58.14
Maximum	28.07	136.11
Mean	19.86	97.16
StdDev	5.62	28.53
N	6	6
50 (35)		
Minimum	12.86	73.36
Maximum	25.97	146.06
Mean	20.75	100.36
StdDev	5.16	24.86
N	6	6

A COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF AMPLITUDE

TIME in minutes		AMPLITUDE in µv.	CHANGE OF AMPLITUDE IN (%) OF CONTROL
55	(40)		
Minimum		13.71	78.21
Maximum		26.00	146.23
Mean		20.56	99.57
StdDev		5.03	24.80
N		6	6
60	(45)		
Minimum		10.20	58.19
Maximum		33.73	152.14
Mean		22.09	105.77
StdDev		8.49	37.94
N		6	6
65	(50)		
Minimum		9.12	52.03
Maximum		32.58	158.38
Mean		21.30	102.10
StdDev		8.92	41.88
N		6	6
70	(55)		
Minimum		8.80	46.73
Maximum		32.69	155.40
Mean		22.16	107.64
StdDev		8.10	40.19
N		6	6
75	(60)	"start wash with Krebs solution"	
Minimum		9.19	48.81
Maximum		29.03	154.61
Mean		22.12	106.88
StdDev		7.31	35.18
N		6	6

A COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF AMPLITUDE

TIME in minutes		AMPLITUDE in µv.	CHANGE OF AMPLITUDE IN (%) OF CONTROL
80	"5"		
Minimum		13.29	70.58
Maximum		31.51	152.98
Mean		23.08	111.09
StdDev		7.49	32.12
N		6	6
85	"10"		
Minimum		14.96	79.45
Maximum		35.38	148.20
Mean		24.78	118.76
StdDev		8.34	31.79
N		6	6
90	"15"		
Minimum		15.03	79.18
Maximum		34.22	176.43
Mean		24.70	119.55
StdDev		8.33	39.04
N		6	6
95	"20"		
Minimum		14.01	74.40
Maximum		34.65	181.83
Mean		24.78	119.61
StdDev		9.26	42.94
N		6	6
100	"25"		
Minimum		13.69	72.43
Maximum		34.40	152.01
Mean		22.35	106.42
StdDev		8.84	32.95
N		6	6

A COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF AMPLITUDE

TIME in minutes		AMPLITUDE in µv.	CHANGE OF AMPLITUDE IN (%) OF CONTROL
105	"30" '10 mM K_2SO_4 started'		
Minimum		13.69	72.70
Maximum		31.71	140.62
Mean		23.94	115.76
StdDev		7.27	31.61
N		6	6
106	'1'		
Minimum		8.12	35.82
Maximum		29.88	132.04
Mean		16.81	83.65
StdDev		8.02	40.72
N		6	6
110	'5'		
Minimum		0.00	0.00
Maximum		8.33	44.24
Mean		2.11	10.65
StdDev		3.14	16.81
N		6	6
115	'10' (wash with Krebs solution)		
Minimum		0.00	0.00
Maximum		0.00	0.00
Mean		0.00	0.00
StdDev		0.00	0.00
N		6	6
116	(1)		
Minimum		6.17	32.77
Maximum		29.26	120.13
Mean		17.01	81.54
StdDev		7.74	33.44
N		6	6

A COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF AMPLITUDE

TIME in minutes	AMPLITUDE in μv.	CHANGE OF AMPLITUDE IN (%) OF CONTROL
120 (5)		
Minimum	11.68	62.03
Maximum	27.86	140.66
Mean	19.29	93.13
StdDev	6.71	30.52
N	6	6
125 (10)		
Minimum	11.78	62.56
Maximum	25.79	145.05
Mean	20.42	100.87
StdDev	5.95	36.43
N	6	6

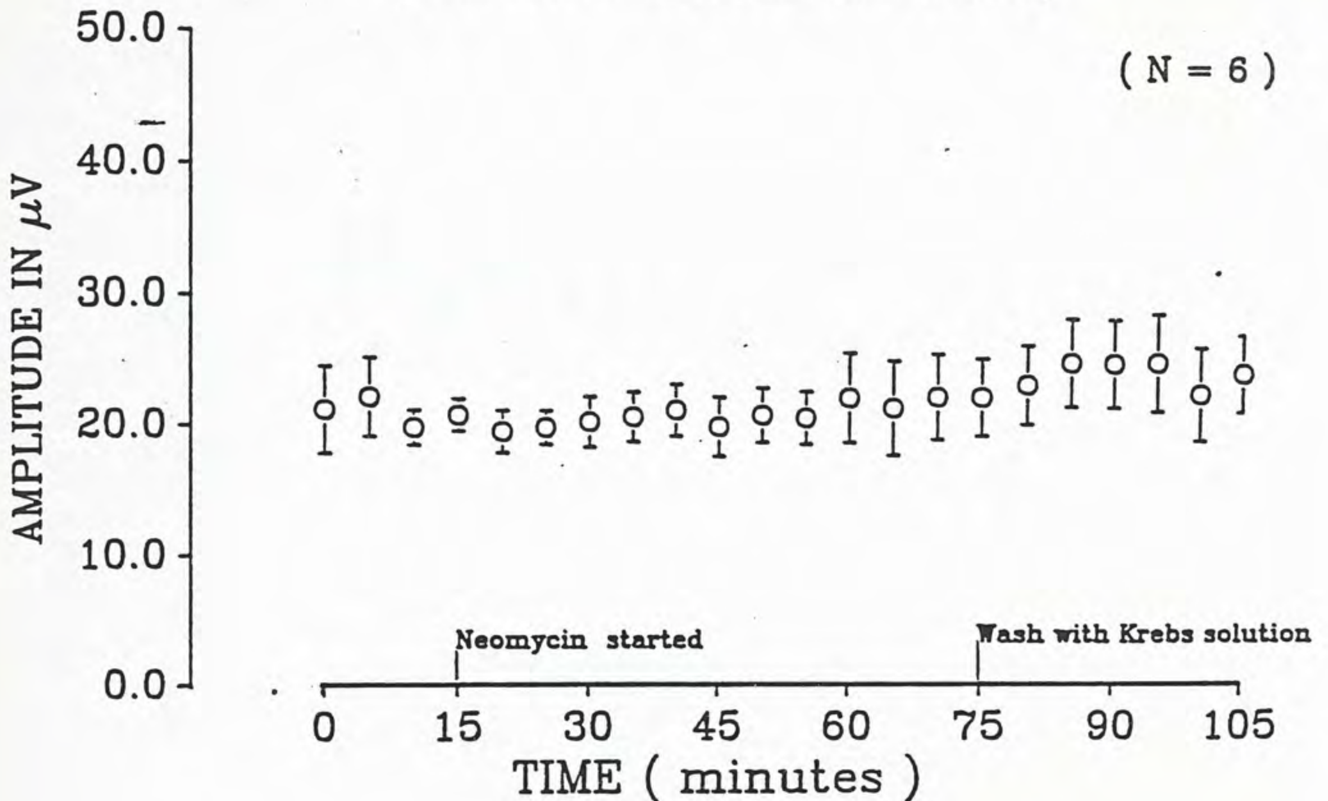


Figure 15 are the data of the A compound action potentials in 6 rat ventrolateral tail nerves. 5.7 mM neomycin solution was started from 15 minutes onward. Whole treatment period was 60 minutes. The nerve was then subsequently washed with Krebs solution. Average potential was plotted against the time. Results were expressed as mean $\pm$SEM. No significant difference from control values was found after the exposure to neomycin solution $(P > 0.05)$.

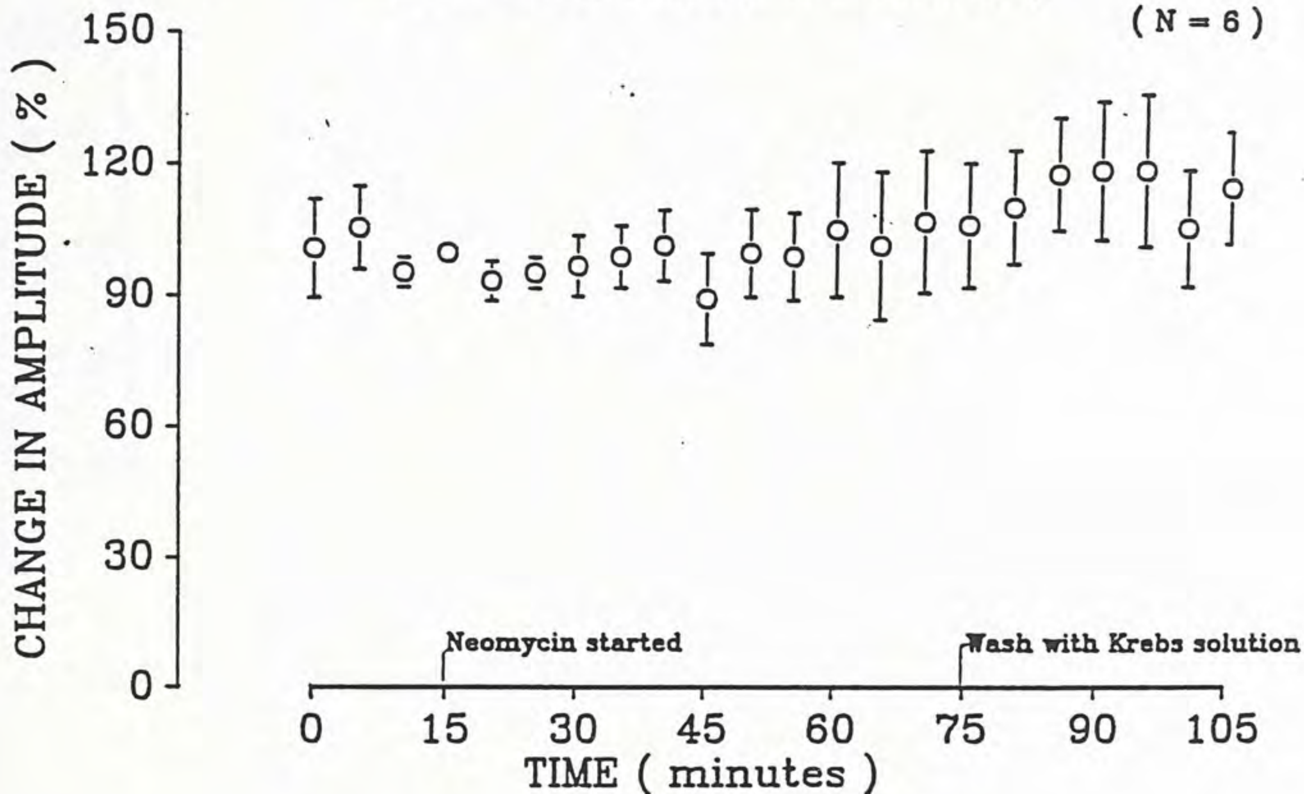


Figure 16 are the data of the A compound action potentials in 6 rat ventrolateral tail nerves. 5.7 mM neomycin solution was started from 15 minutes onward. Whole treatment period was 60 minutes. The nerve was then subsequently washed with Krebs solution. The amplitude before the application of neomycin was taken as 100%. Change in amplitude was plotted against the time. Results were expressed as mean ± SEM. No significant difference from control values was found after the exposure to neomycin solution ($P > 0.05$).

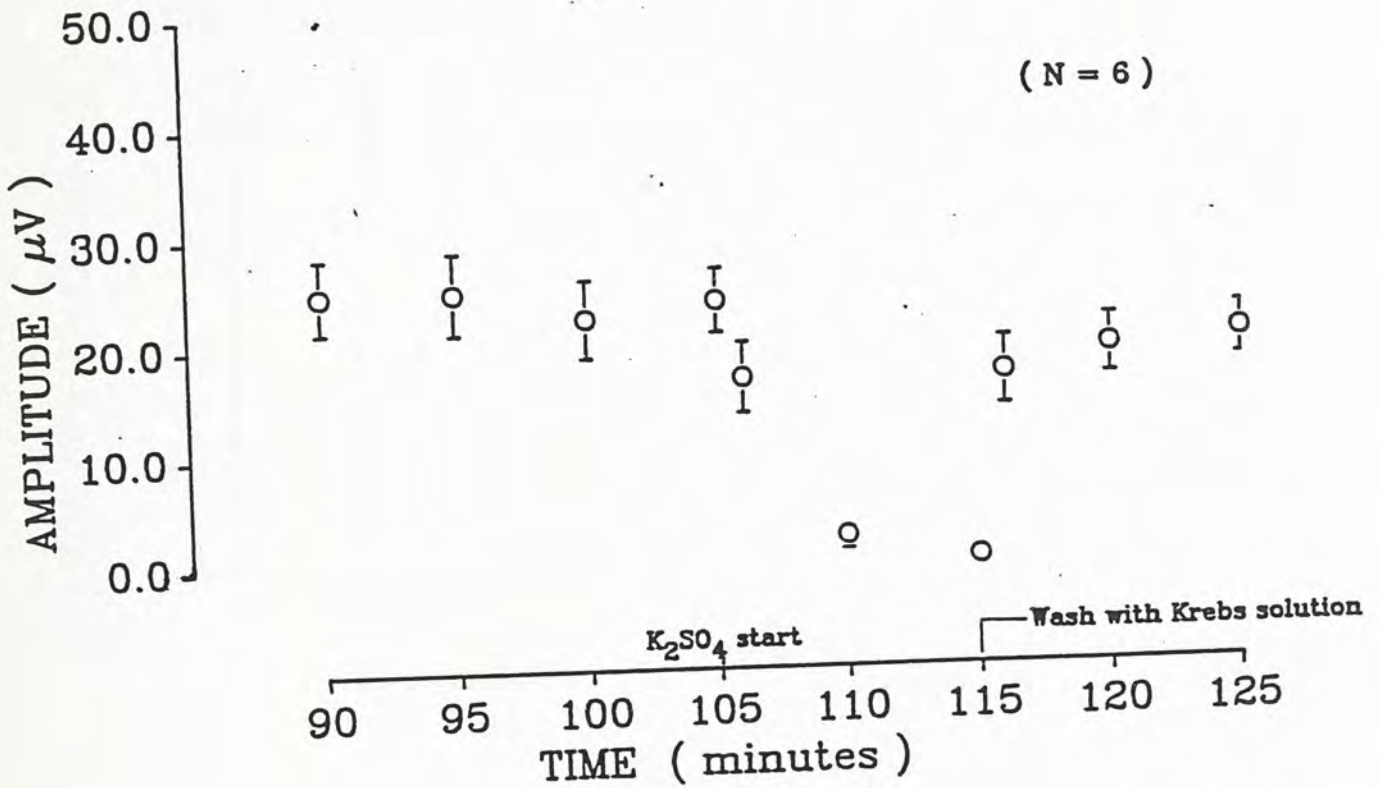


Figure 19 are the data of the A compound action potentials in 6 rat ventrolateral tail nerves treated with 10 mM K_2SO_4 Krebs solution. Complete blockade was shown from 5 minutes onward after the application of K_2SO_4 solution . The nerve was subsequently washed with Krebs solution. Amplitude was plotted against the time. Results were expressed as mean $\pm$SEM. Significant difference from control values was found at 5 and 10 minutes after the exposure to 10 mM K_2SO_4 Krebs solution ($P \leq 0.05$).

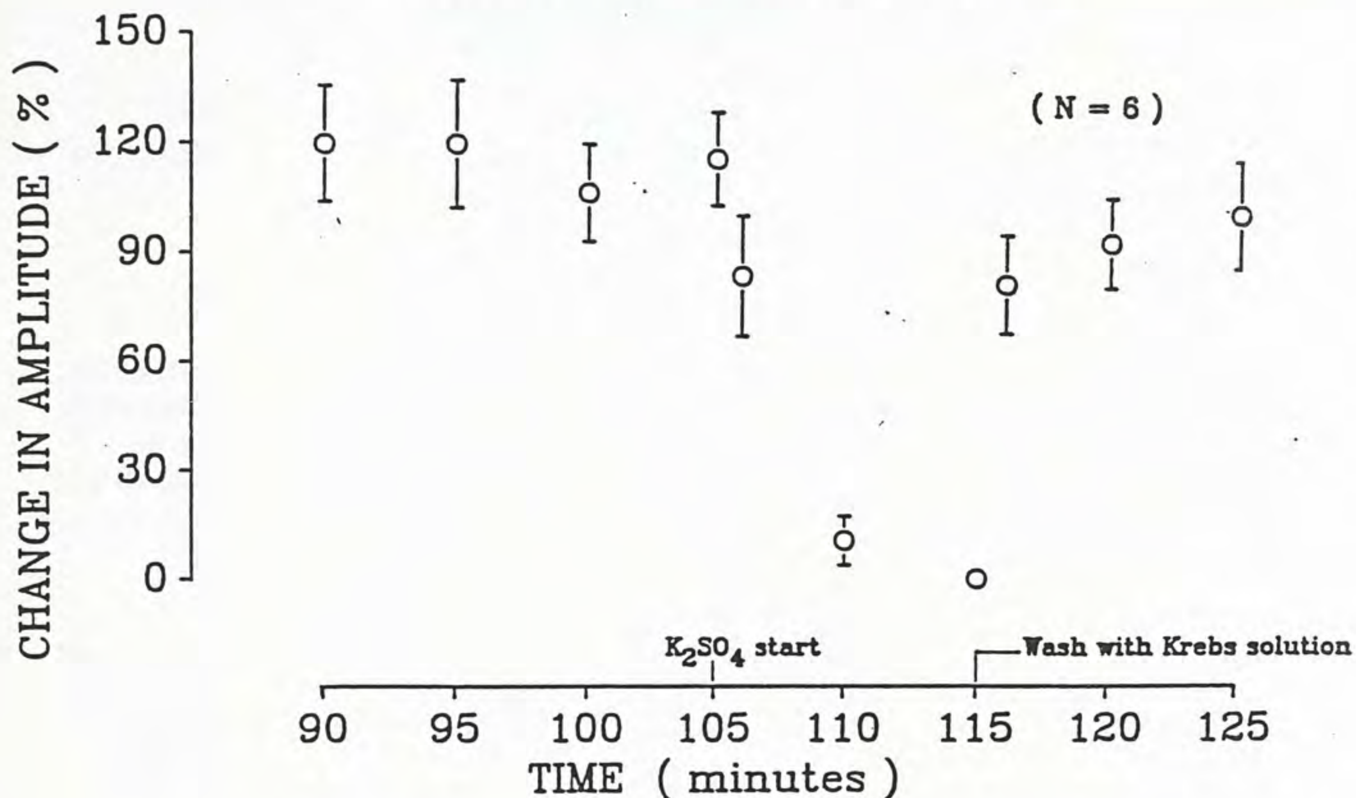


Figure 20 are the data of the A compound action potentials in 6 rat ventrolateral tail nerve treated with 10 mM K_2SO_4 Krebs solution. Complete blockade was shown from 5 minutes onward. The nerve was subsequently washed with Krebs solution. The amplitude before the application of K_2SO_4 was taken as 100%. Change in amplitude was plotted against the time. Results were expressed as mean $\pm$ SEM. Significant difference from control values was found at 5 and 10 minutes after the exposure to 10 mM K_2SO_4 Krebs solution ($P \leq 0.05$).

TABLE 13

A COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF CONDUCTION VELOCITY

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
0		
Minimum	21.0	92.95
Maximum	27.7	111.11
Mean	24.2	97.97
StdDev	3.0	6.80
N	6	6
5		
Minimum	19.6	84.26
Maximum	27.8	106.38
Mean	23.6	95.24
StdDev	3.8	8.91
N	6	6
10		
Minimum	20.7	92.95
Maximum	27.8	112.62
Mean	24.7	100.16
StdDev	2.7	7.17
N	6	6
15 (Neomycin started)		
Minimum	22.1	100.00
Maximum	27.8	100.00
Mean	24.7	100.00
StdDev	2.0	.00
N	6	6
20 (5)		
Minimum	22.1	95.73
Maximum	26.6	106.03
Mean	24.4	99.01
StdDev	1.7	3.77
N	6	6

A COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF CONDUCTION VELOCITY

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
25 (10)		
Minimum	21.0	91.81
Maximum	27.3	104.38
Mean	24.3	98.58
StdDev	2.2	5.06
N	6	6
30 (15)		
Minimum	20.7	91.81
Maximum	34.7	138.89
Mean	26.2	106.39
StdDev	4.6	17.02
N	6	6
35 (20)		
Minimum	20.3	91.81
Maximum	26.6	108.08
Mean	24.6	100.01
StdDev	2.2	6.97
N	6	6
40 (25)		
Minimum	20.0	90.38
Maximum	26.2	108.08
Mean	24.3	98.43
StdDev	2.4	6.32
N	6	6
45 (30)		
Minimum	19.7	80.31
Maximum	27.1	110.30
Mean	23.7	96.20
StdDev	3.2	12.52
N	6	6

A COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF CONDUCTION VELOCITY

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
50 (35)		
Minimum	19.7	88.96
Maximum	27.7	111.11
Mean	24.7	99.87
StdDev	3.1	9.15
N	6	6
55 (40)		
Minimum	21.4	92.95
Maximum	28.4	113.64
Mean	24.8	100.49
StdDev	2.6	8.42
N	6	6
60 (45)		
Minimum	21.0	83.29
Maximum	27.1	108.70
Mean	24.1	98.16
StdDev	2.1	8.98
N	6	6
65 (50)		
Minimum	21.0	80.31
Maximum	27.7	111.11
Mean	23.8	96.75
StdDev	2.9	10.93
N	6	6
70 (55)		
Minimum	21.2	76.21
Maximum	27.1	110.30
Mean	23.9	97.19
StdDev	2.7	12.55
N	6	6

A COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF CONDUCTION VELOCITY

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
75 (60) "start wash with Krebs solution"		
Minimum	21.4	78.89
Maximum	26.3	112.62
Mean	23.5	95.57
StdDev	2.0	10.90
N	6	6
80 "5"		
Minimum	21.0	78.89
Maximum	26.6	110.30
Mean	23.9	97.25
StdDev	2.5	11.15
N	6	6
85 "10"		
Minimum	20.3	73.71
Maximum	30.4	121.95
Mean	24.7	100.49
StdDev	4.5	17.66
N	6	6
90 "15"		
Minimum	21.2	76.21
Maximum	32.0	128.21
Mean	25.8	104.95
StdDev	4.5	17.75
N	6	6
95 "20"		
Minimum	19.7	74.94
Maximum	31.2	125.00
Mean	25.0	101.70
StdDev	4.8	18.40
N	6	6

A COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF CONDUCTION VELOCITY

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
100 "25"		
Minimum	20.0	74.94
Maximum	29.9	116.28
Mean	24.3	98.62
StdDev	4.3	16.25
N	6	6
105 "30" '10 mM K_2SO_4 started'		
Minimum	19.9	73.71
Maximum	29.9	119.05
Mean	24.3	98.89
StdDev	4.5	17.47
N	6	6
106 '1'		
Minimum	17.1	68.49
Maximum	27.3	110.30
Mean	21.5	87.46
StdDev	4.2	16.29
N	6	6
110 '5'		
Minimum	0.0	0.00
Maximum	25.7	110.30
Mean	14.3	59.08
StdDev	8.6	38.05
N	6	6
115 '10' (wash with Krebs solution)		
Minimum	0.0	0.00
Maximum	0.0	0.00
Mean	0.0	0.00
StdDev	0.0	0.00
N	6	6

A COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF CONDUCTION VELOCITY

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
116 (1)		
Minimum	14.0	56.18
Maximum	25.2	108.08
Mean	18.5	76.15
StdDev	3.8	19.73
N	6	6
120 (5)		
Minimum	14.0	56.18
Maximum	27.3	112.62
Mean	21.6	88.05
StdDev	4.8	20.05
N	6	6
125 (10)		
Minimum	14.0	56.18
Maximum	27.3	115.04
Mean	21.9	89.13
StdDev	4.9	20.49
N	6	6

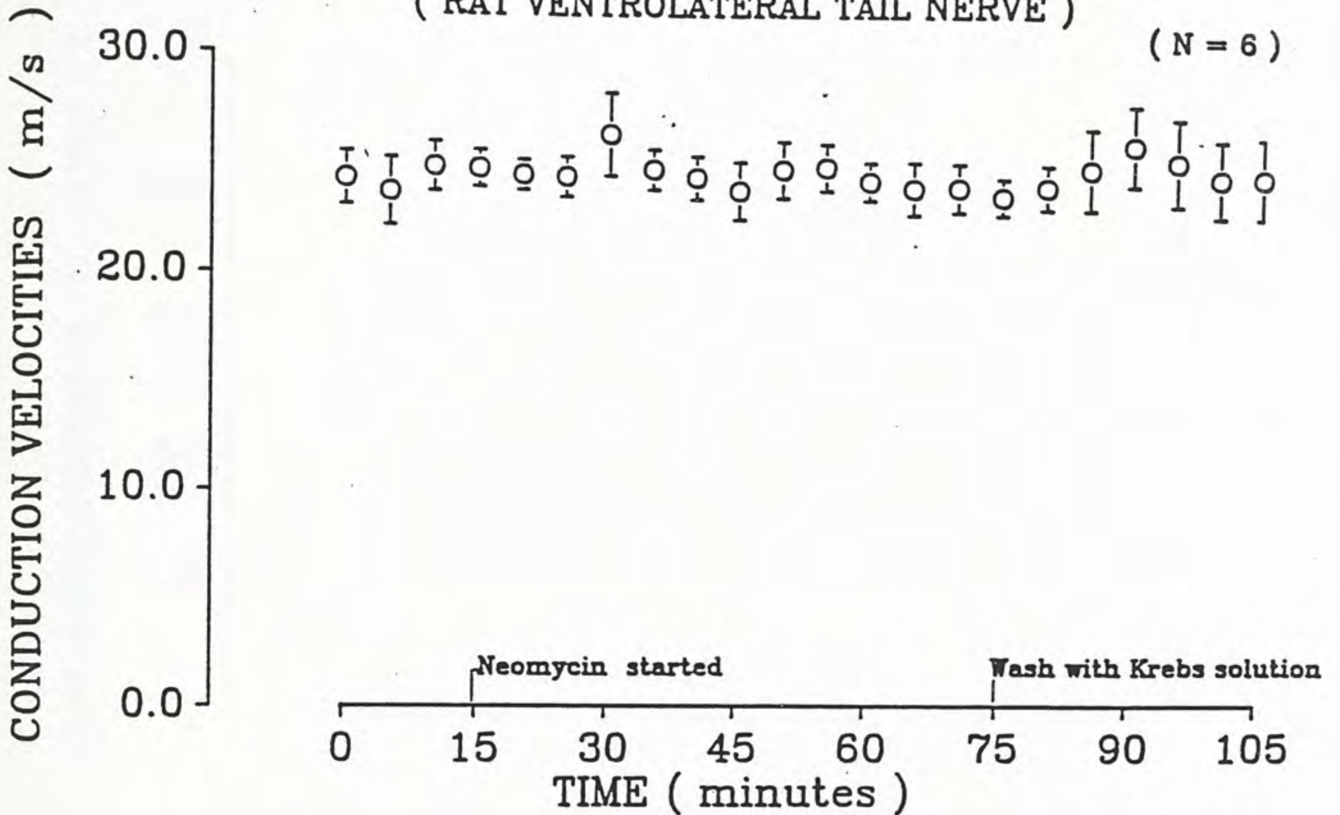


Figure 17 are the data of the A compound action potentials in 6 rat ventrolateral tail nerves. 5.7 mM neomycin solution was started from 15 minutes onward. Whole treatment period was 60 minutes. The nerve was then subsequently washed with Krebs solution. Conduction velocity was plotted against the time. Results were expressed as mean $\pm$SEM. No significant difference from control values was found after the exposure to neomycin solution ($P > 0.05$).

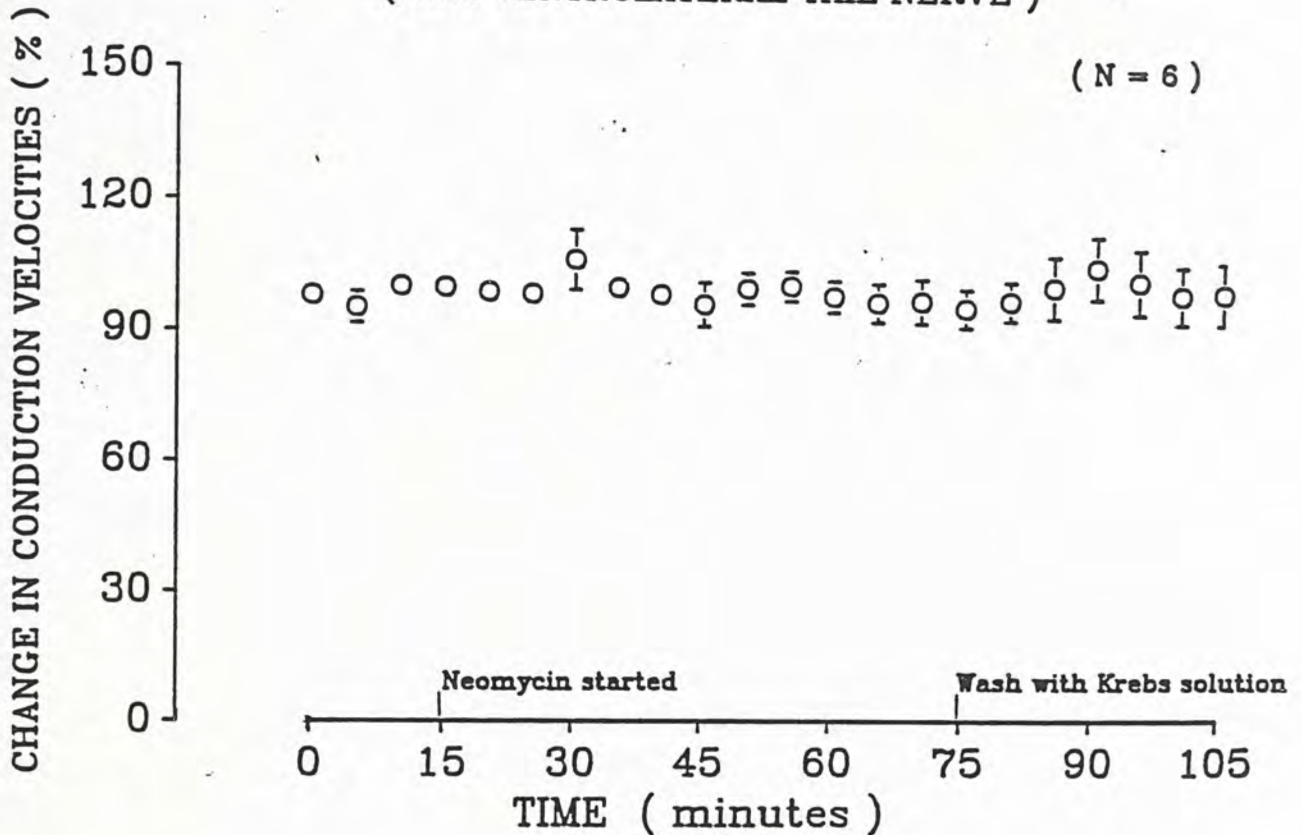


Figure 18 are the data of the A compound action potentials in 6 rat ventrolateral tail nerves. 5.7 mM neomycin solution was started from 15 minutes onward. Whole treatment period was 60 minutes. The nerve was then subsequently washed with Krebs solution. The conduction velocity before the application of neomycin solution was taken as 100 %. Change in conduction velocity was plotted against the time. Results were expressed as mean $\pm$SEM. No significant difference from contol values was found after the exposure to neomycin solution ($P > 0.05$).

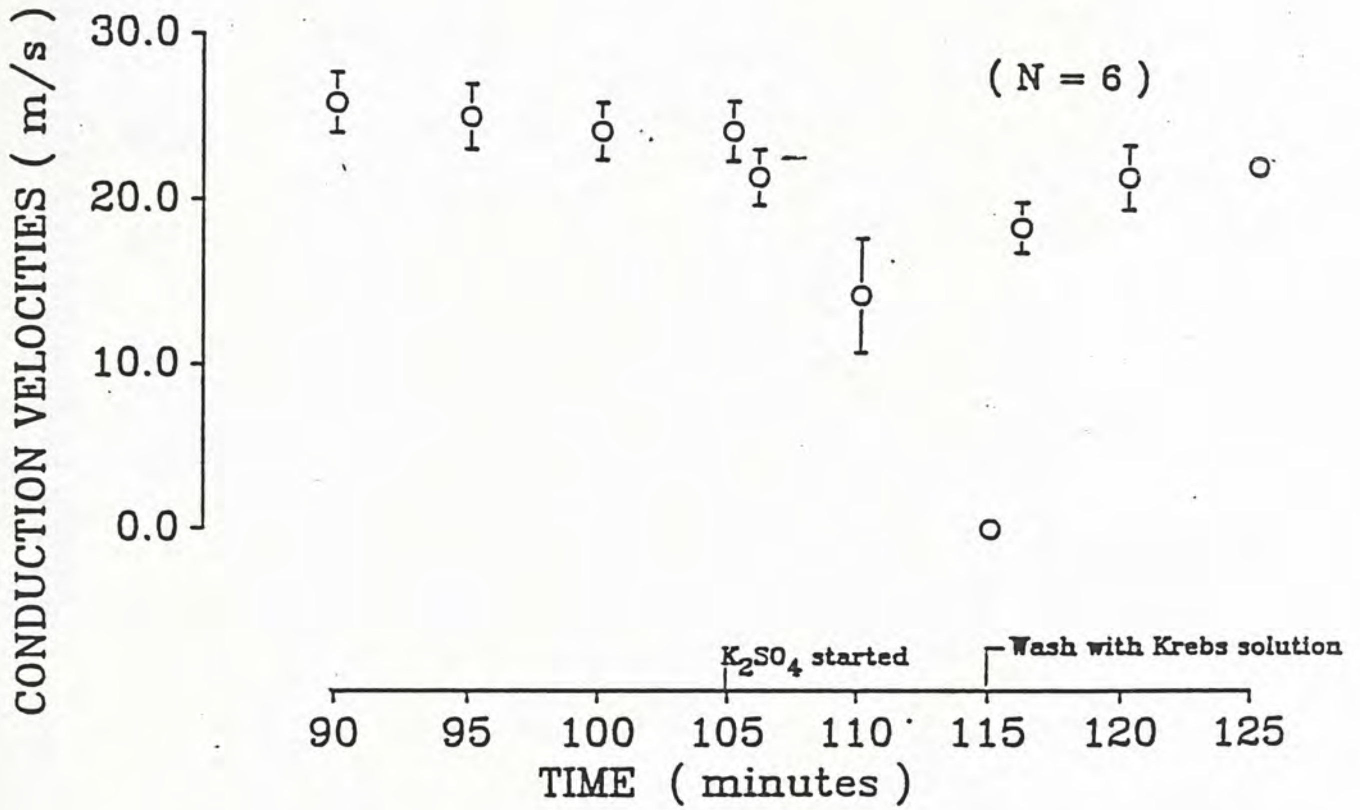


Figure 21 are the data of the A compound action potentials in 6 rat ventrolateral tail nerves treated with 10 mM K_2SO_4 Krebs solution. The nerve was subsequently washed with Krebs solution. Conduction velocity was plotted against the time. Results were expressed as mean $\pm$SEM. Significant difference from control values was found at 5 and 10 minutes after the exposure to 10 mM K_2SO_4 Krebs solution ($P \leq 0.05$).

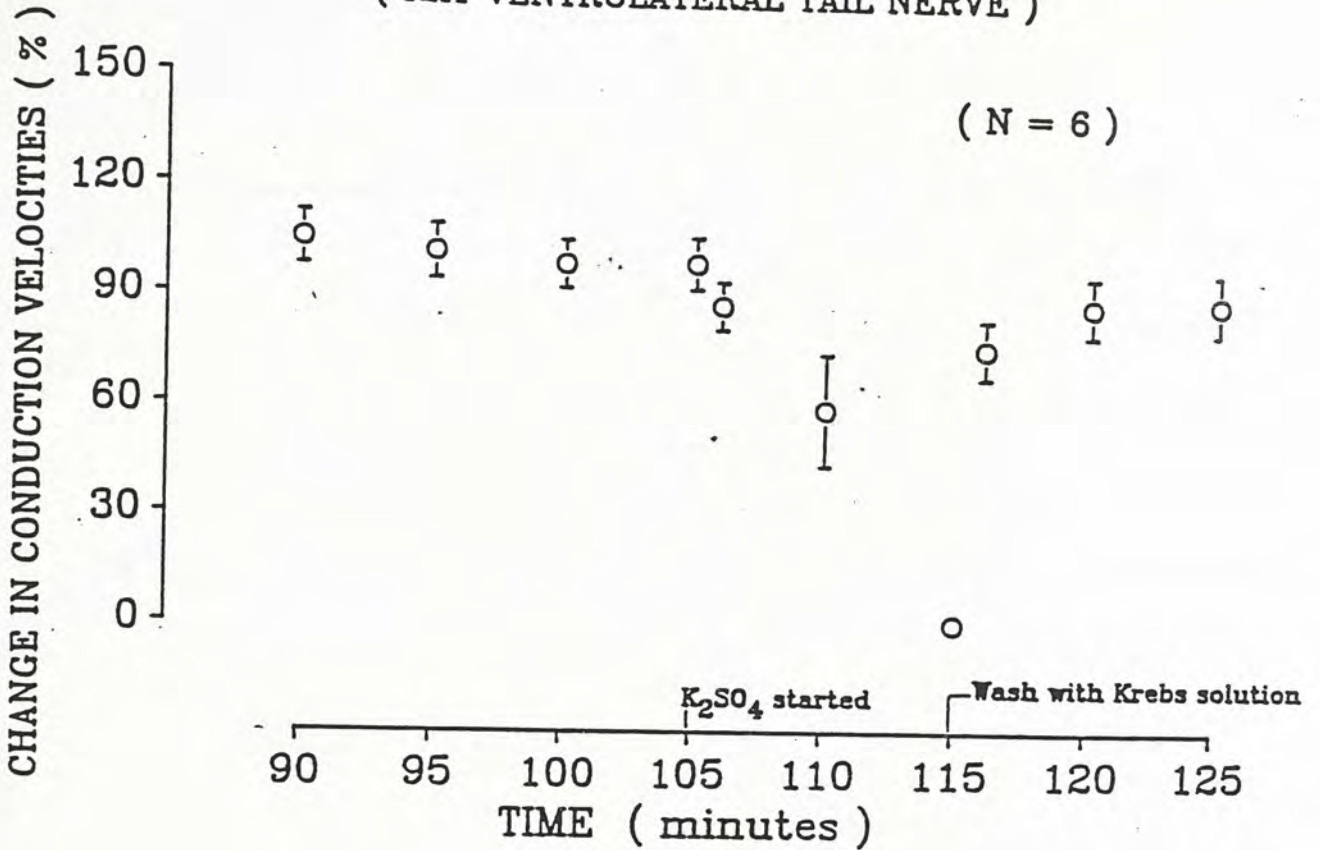


Figure 22 are the data of the A compound action potentials in 6 rat ventrolateral tail nerves treated with 10 mM K_2SO_4 Krebs solution. The nerve was subsequently washed with Krebs solution. The conduction velocity before the application of K_2SO_4 was taken as 100%. Change in conduction velocity was plotted against the time. Results were expressed as mean $\pm$SEM. Significant difference from control values was found at 5 and 10 minutes after the exposure to 10 mM K_2SO_4 Krebs solution ($P \leq 0.05$).

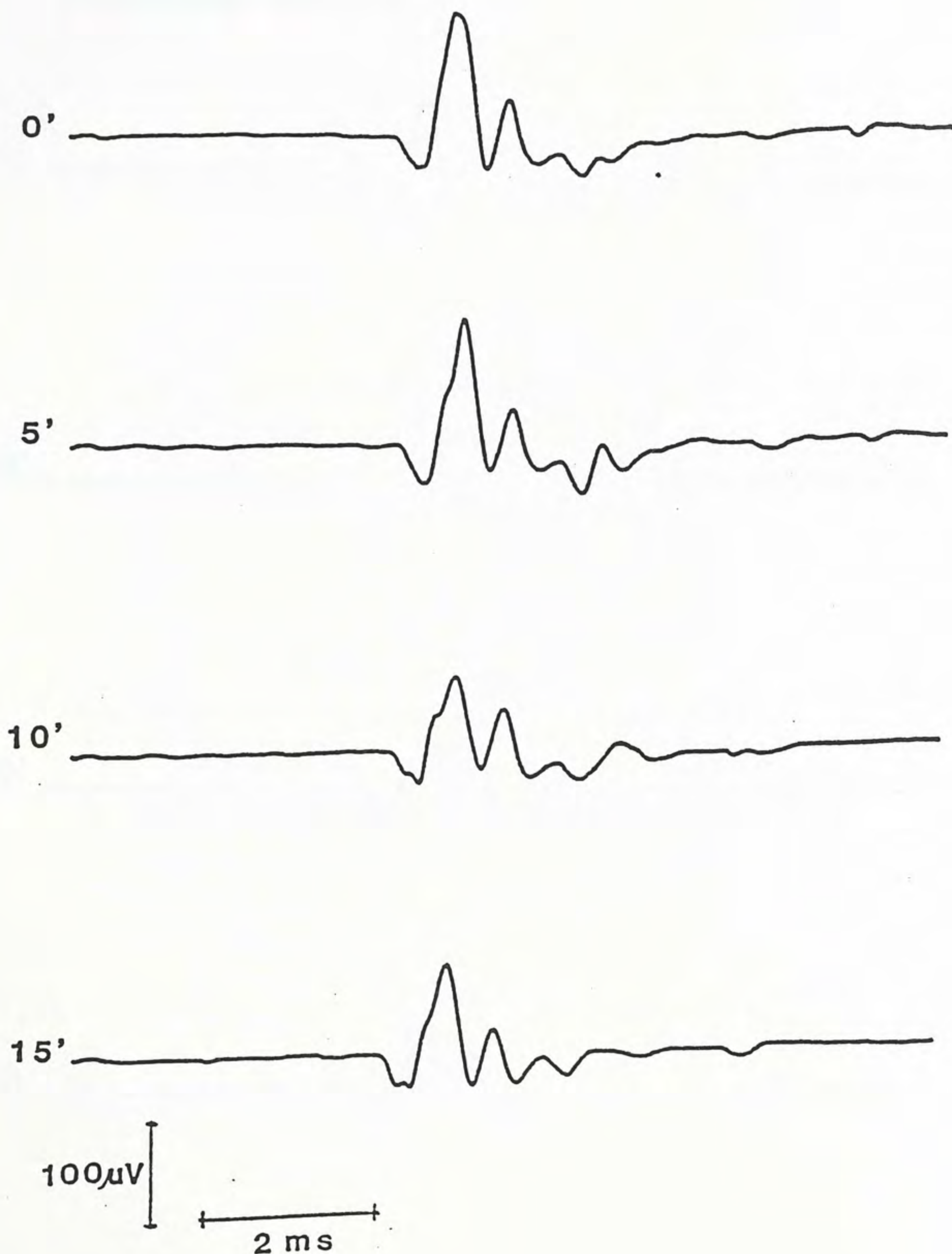


Figure 23 In vivo study of A compound action potential in rat ventrolateral tail nerve, original recording of the action potential with exposure to 5.7 mM neomycin solution in Krebs solution for 60 minutes, washed with Krebs solution for 30 minutes and subsequently expose with 10 mM K_2SO_4 to shown the blockade effect. Conductive distance was 8.6 cm.

Neomycin started

40'

45'

50'

55'

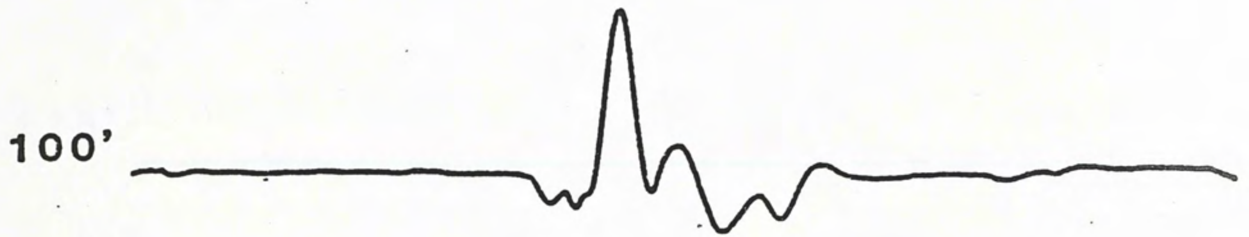
60'

65'

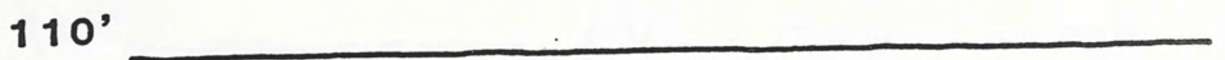
70'

75'

Wash with Krebs solution



10 mM K_2SO_4 started



115'

Wash with Krebs solution

116'

120'

125'

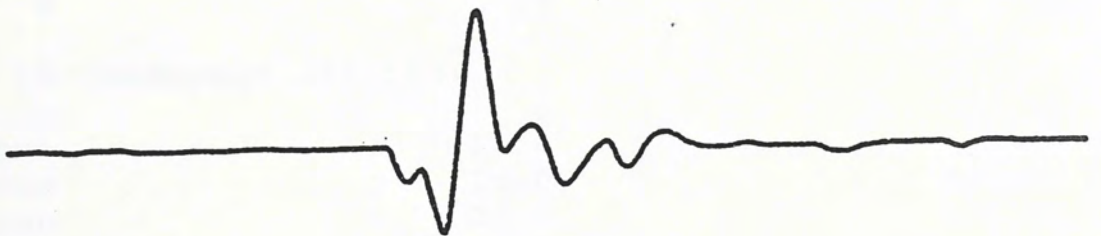


Figure 3. (continued)

TABLE 9

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
NEOMYCIN STUDIES

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
0		
Minimum	18.8	94.02
Maximum	25.0	100.00
Mean	21.5	99.57
StdDev	1.9	1.60
N	14	14
5		
Minimum	18.8	94.02
Maximum	25.0	100.00
Mean	21.5	99.57
StdDev	1.9	1.60
N	14	14
10		
Minimum	18.8	100.00
Maximum	25.0	100.00
Mean	21.6	100.00
StdDev	1.9	0.00
N	14	14
15 (Neomycin started)		
Minimum	18.8	100.00
Maximum	25.0	100.00
Mean	21.6	100.00
StdDev	1.9	0.00
N	14	14
16 (1)		
Minimum	18.8	90.87
Maximum	25.0	100.00
Mean	21.4	99.35
StdDev	1.9	2.44
N	14	14

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
NEOMYCIN STUDIES

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
17 (2)		
Minimum	18.8	90.87
Maximum	25.0	105.13
Mean	21.5	99.71
StdDev	1.8	2.89
N	14	14
18 (3)		
Minimum	18.8	90.87
Maximum	25.0	100.00
Mean	21.4	99.35
StdDev	1.9	2.44
N	14	14
19 (4)		
Minimum	18.8	90.87
Maximum	25.0	100.00
Mean	21.4	99.35
StdDev	1.9	2.44
N	14	14
20 (5)		
Minimum	18.8	90.87
Maximum	25.0	100.00
Mean	21.4	99.35
StdDev	1.9	2.44
N	14	14
21 (6)		
Minimum	18.8	90.87
Maximum	25.0	105.13
Mean	21.4	99.35
StdDev	1.8	3.15
N	14	14

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
NEOMYCIN STUDIES

TIME in minutes	CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
22 (7)		
Minimum	18.8	94.94
Maximum	25.0	105.13
Mean	21.5	99.70
StdDev	1.8	2.30
N	14	14
23 (8)		
Minimum	17.6	93.81
Maximum	25.0	105.13
Mean	21.4	99.26
StdDev	1.9	2.78
N	14	14
24 (9)		
Minimum	17.6	93.81
Maximum	25.0	105.13
Mean	21.4	99.26
StdDev	1.9	2.78
N	14	14
25 (10)		
Minimum	17.6	93.81
Maximum	25.0	105.13
Mean	21.4	99.26
StdDev	1.9	2.78
N	14	14
26 (11)		
Minimum	18.8	94.94
Maximum	25.0	105.13
Mean	21.6	100.24
StdDev	1.7	2.18
N	14	14

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
NEOMYCIN STUDIES

TIME in minutes		CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
27	(12)		
Minimum		17.6	93.81
Maximum		25.0	105.13
Mean		21.5	99.56
StdDev		2.0	2.59
N		14	14
28	(13)		
Minimum		18.8	94.94
Maximum		25.0	105.13
Mean		21.6	100.00
StdDev		1.8	2.00
N		14	14
29	(14)		
Minimum		18.8	94.94
Maximum		25.0	105.13
Mean		21.6	100.00
StdDev		1.8	2.00
N		14	14
30	(15)		
Minimum		17.6	93.81
Maximum		25.0	105.13
Mean		21.5	99.56
StdDev		2.0	2.59
N		14	14
31	(16)		
Minimum		18.8	94.94
Maximum		25.0	106.19
Mean		21.6	100.45
StdDev		1.7	2.59
N		14	14

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
NEOMYCIN STUDIES

TIME in minutes		CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
32	(17)		
Minimum		18.8	94.94
Maximum		25.0	100.00
Mean		21.4	99.36
StdDev		1.9	1.65
N		14	14
33	(18)		
Minimum		18.8	94.94
Maximum		25.0	105.13
Mean		21.6	100.00
StdDev		1.8	2.00
N		14	14
34	(19)		
Minimum		18.8	94.94
Maximum		25.0	105.13
Mean		21.6	100.00
StdDev		1.8	2.00
N		14	14
35	(20)		
Minimum		18.8	94.94
Maximum		25.0	105.13
Mean		21.6	100.00
StdDev		1.8	2.00
N		14	14
36	(21)		
Minimum		18.8	94.39
Maximum		25.0	105.13
Mean		21.5	99.60
StdDev		1.9	2.50
N		14	14

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
NEOMYCIN STUDIES

TIME in minutes		CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
37	(22)		
Minimum		18.8	94.94
Maximum		25.0	105.13
Mean		21.6	100.00
StdDev		1.8	2.00
N		14	14
38	(23)		
Minimum		18.8	94.94
Maximum		25.0	105.13
Mean		21.6	100.00
StdDev		1.8	2.00
N		14	14
39	(24)		
Minimum		18.8	94.94
Maximum		25.0	100.00
Mean		21.5	99.64
StdDev		1.8	1.35
N		14	14
40	(25)		
Minimum		18.8	94.94
Maximum		25.0	100.00
Mean		21.5	99.64
StdDev		1.8	1.35
N		14	14
41	(26)		
Minimum		18.8	94.94
Maximum		25.0	100.00
Mean		21.5	99.64
StdDev		1.8	1.35
N		14	14

A COMPOUND ACTION POTENTIAL
FROG SCIATIC NERVE
DATA OF CONDUCTION VELOCITY
NEOMYCIN STUDIES

TIME in minutes		CONDUCTION VELOCITY in m/s	CHANGE OF CONDUCTION VELOCITY IN (%) OF CONTROL
42	(27)		
Minimum		18.8	94.94
Maximum		25.0	100.00
Mean		21.5	99.64
StdDev		1.8	1.35
N		14	14
43	(28)		
Minimum		18.8	94.94
Maximum		25.0	100.00
Mean		21.5	99.64
StdDev		1.8	1.35
N		14	14
44	(29)		
Minimum		18.8	94.94
Maximum		25.0	100.00
Mean		21.5	99.64
StdDev		1.8	1.35
N		14	14
45	(30)		
Minimum		18.8	94.94
Maximum		25.0	103.24
Mean		21.5	99.87
StdDev		1.8	1.66
N		14	14

STATISTICAL ANALYSIS

There was significant difference in change of amplitudes and conduction velocities from control at 5 and 10 minutes after expsoure to 10 mM K_2SO_4 in Krebs solution ($p < 0.05$). No significant difference was found in change in amplitude and conduction velocity before and after the infusion of neomycin in Krebs solution.

2.3 RAT VENTROLATERAL TAIL NERVE:- C COMPOUND ACTION POTENTIAL

AMPLITUDES OF C POTENTIAL:-

Table 14 lists the minimum, maximum, mean and standard deviation of the C potentials from ventrolateral tail nerves of 9 rats. Integrated potential was computed. The change in amplitude with time was plotted. Figure 24 represents the change in amplitudes with time. Results were expressed as mean $\pm$ SEM. There was no linear relationship between change in potential with time. The mean value before the start of neomycin infusion was 4.02 $\pm$0.48 uV The mean value at the end of the treatment was 4.63 $\pm$0.63 uV. No apparent change was found when the nerve was washed with Krebs solution. When the values obtained before the start of the neomycin infusion was 100%, the change in amplitudes are shown in figure 25.

CONDUCTION VELOCITIES

Table 15 lists the minimum, maximum, mean and standard deviation of the conduction velocities from ventrolateral tail nerves of 9 rats. Conduction velocities of the fastest and the slowest C potential were recorded. The change in conduction velocities with time was plotted. Figure 26 represents the change in conduction velocities with time. Results were expressed as mean $\pm$SEM. The mean value before the infusion of

neomycin solution were 0.89 ±0.04 and 0.54 ±0.02 m/s respectively. At the end of the treatment, the mean value were 0.96 ±0.07 and 0.64 ±0.04 m/s respectively. There were no significant difference between change in conduction velocities with time ($p > 0.05$). No apparent change was found when the nerve was washed with Krebs solution. When the values obtained before the start of the neomycin infusion was 100%, the change in conduction velocities are shown in figure 27.

10 mM POTASSIUM SULPHATE KREBS SOLUTION

AMPLITUDES OF C POTENTIAL

Table 14 lists the minimum, maximum, mean and standard deviation of the amplitudes from ventrolateral tail nerves of 9 rats. Integrated potential was computed. The change in amplitudes with the time was plotted. Figure 28 represents the change in amplitudes with time. Results were expressed as mean ±SEM. The mean value before the infusion of 10 mM K_2SO_4 was 4.41 ±0.55 uV . At the end of the treatment, the mean values was 0.80 ±0.21 uV. Significant difference in change of amplitude from control was found at 10 and 15 minutes after exposure to 10 mM K_2SO_4 Krebs solution ($p \leq 0.05$). When the nerve was flushed with Krebs solution, there were gradual return of the amplitude of the potential. The mean value at the end of the treatment were 3.18 ±0.55 uV. When the value

obtained before the start of potassium sulphate infusion was taken as 100%, the change in potentials are shown in figure 29.

CONDUCTION VELOCITIES

Table 15 lists the minimum, maximum, mean and standard deviation of the conduction velocities from ventrolateral tail nerve of 9 rats. Conduction velocities of the fastest and slowest C potential were recorded. Figure 30 represents the change in conduction velocities with time. Results were expressed as mean $\pm$ SEM. The mean values at the beginning of the treatment were 0.95 $\pm$0.04 and 0.65 $\pm$0.05 m/s respectively. At the end of the 10 mM K_2SO_4 treatment, the mean values were 0.45 $\pm$0.07 and 0.36 $\pm$0.05 m/s respectively. Significant difference from control was found at 15 minutes after exposure to 10 mM K_2SO_4 Krebs solution ($p < 0.05$). When the nerve was flushed with Krebs solution, there were gradual return of the conduction velocities. At 15 minutes, the mean values were 0.89 $\pm$0.07 and 0.60 $\pm$0.03 m/s respectively. When the values obtained before the start of the potassium sulphate were taken as 100%, the change in conduction velocities are shown in figure 31.

Figure 32 and 33 are two sets of original recordings of the C compound action potential on the effect of neomycin and 10 mM K_2SO_4 Krebs solution.

TABLE 14

C COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF AMPLITUDE

TIME in minutes	INTERGRATED AMPLITUDE in µV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
0		
Minimum	2.02	78.08
Maximum	6.70	125.70
Mean	3.83	95.54
StdDev	1.50	14.97
N	9	9
5		
Minimum	1.70	69.96
Maximum	6.46	135.71
Mean	4.03	102.43
StdDev	1.40	22.77
N	9	9
10		
Minimum	2.71	76.99
Maximum	6.20	143.33
Mean	4.03	103.05
StdDev	1.36	19.19
N	9	9
15 (Neomycin started)		
Minimum	2.10	100.00
Maximum	6.56	100.00
Mean	4.02	100.00
StdDev	1.43	0.00
N	9	9
20 (5)		
Minimum	1.74	82.86
Maximum	6.19	120.29
Mean	3.97	98.51
StdDev	1.43	11.46
N	9	9

C COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF AMPLITUDE

TIME in minutes	INTERGRATED AMPLITUDE in μV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
25 (10)		
Minimum	1.54	73.33
Maximum	7.25	126.29
Mean	4.01	99.04
StdDev	1.63	16.29
N	9	9
30 (15)		
Minimum	1.45	69.05
Maximum	5.85	134.19
Mean	4.07	101.61
StdDev	1.43	19.58
N	9	9
35 (20)		
Minimum	1.71	81.43
Maximum	7.35	119.09
Mean	4.27	103.87
StdDev	1.81	12.60
N	9	9
40 (25)		
Minimum	1.65	75.31
Maximum	6.82	123.26
Mean	4.19	100.49
StdDev	1.93	17.52
N	9	9
45 (30)		
Minimum	1.77	62.96
Maximum	6.26	117.45
Mean	3.84	96.38
StdDev	1.38	17.18
N	9	9
50 (35)		
Minimum	1.57	74.76
Maximum	5.30	111.33
Mean	3.68	90.74
StdDev	1.39	11.80
N	9	9

C COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF AMPLITUDE

TIME in minutes	INTERGRATED AMPLITUDE in μV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
55 (40)		
Minimum	1.21	57.62
Maximum	5.95	143.14
Mean	4.02	99.57
StdDev	1.53	25.14
N	9	9
60 (45)		
Minimum	1.59	75.15
Maximum	6.97	135.08
Mean	4.40	107.95
StdDev	1.85	23.43
N	9	9
65 (50)		
Minimum	2.06	85.19
Maximum	7.13	138.18
Mean	4.46	109.51
StdDev	1.78	16.91
N	9	9
70 (55)		
Minimum	1.94	79.84
Maximum	6.72	135.61
Mean	4.56	112.36
StdDev	1.70	15.33
N	9	9
75 (60) "start wash with Krebs solution"		
Minimum	2.29	91.48
Maximum	7.07	134.69
Mean	4.63	113.71
StdDev	1.88	15.38
N	9	9

C COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF AMPLITUDE

TIME in minutes		INTERGRATED AMPLITUDE in µV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
80	"5"		
Minimum		2.07	86.83
Maximum		7.02	136.05
Mean		4.59	111.75
StdDev		1.95	17.15
N		9	9
85	"10"		
Minimum		2.14	91.76
Maximum		6.75	130.81
Mean		4.42	108.98
StdDev		1.70	11.81
N		9	9
90	"15"		
Minimum		2.00	81.10
Maximum		7.53	145.93
Mean		4.31	107.55
StdDev		1.68	22.23
N		9	9
95	"20"		
Minimum		1.89	81.07
Maximum		6.91	133.91
Mean		4.34	106.57
StdDev		1.75	20.92
N		9	9
100	"25"		
Minimum		1.88	74.85
Maximum		6.73	130.43
Mean		4.34	108.51
StdDev		1.59	18.70
N		9	9

C COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF AMPLITUDE

TIME in minutes	INTERGRATED AMPLITUDE in μV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
105 "30" '10 mM K_2SO_4 started'		
Minimum	1.73	78.05
Maximum	6.52	148.57
Mean	4.42	110.34
StdDev	1.65	23.42
N	9	9
106 '1'		
Minimum	1.34	46.88
Maximum	4.30	88.10
Mean	2.55	65.46
StdDev	.90	16.64
N	9	9
110 '5'		
Minimum	.30	8.57
Maximum	4.65	106.67
Mean	2.31	58.19
StdDev	1.27	26.22
N	9	9
115 '10'		
Minimum	.02	.57
Maximum	3.04	57.04
Mean	1.38	32.28
StdDev	.98	15.22
N	9	9
120 '15' (wash with Krebs solution)		
Minimum	0.00	0.00
Maximum	1.76	33.02
Mean	.80	17.67
StdDev	.62	11.16
N	9	9

C COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF AMPLITUDE

TIME in minutes	INTERGRATED AMPLITUDE in μV	CHANGE OF AMPLITUDE IN (%) OF CONTROL
121 (1)		
Minimum	.87	24.86
Maximum	3.50	75.24
Mean	2.06	52.47
StdDev	.93	17.17
N	9	9
125 (5)		
Minimum	.61	17.43
Maximum	5.12	96.06
Mean	2.75	67.06
StdDev	1.47	21.57
N	9	9
130 (10)		
Minimum	.61	17.43
Maximum	5.78	108.44
Mean	2.93	69.71
StdDev	1.75	24.52
N	9	9
135 (15)		
Minimum	.61	17.43
Maximum	5.45	102.06
Mean	3.18	77.35
StdDev	1.66	23.99
N	9	9

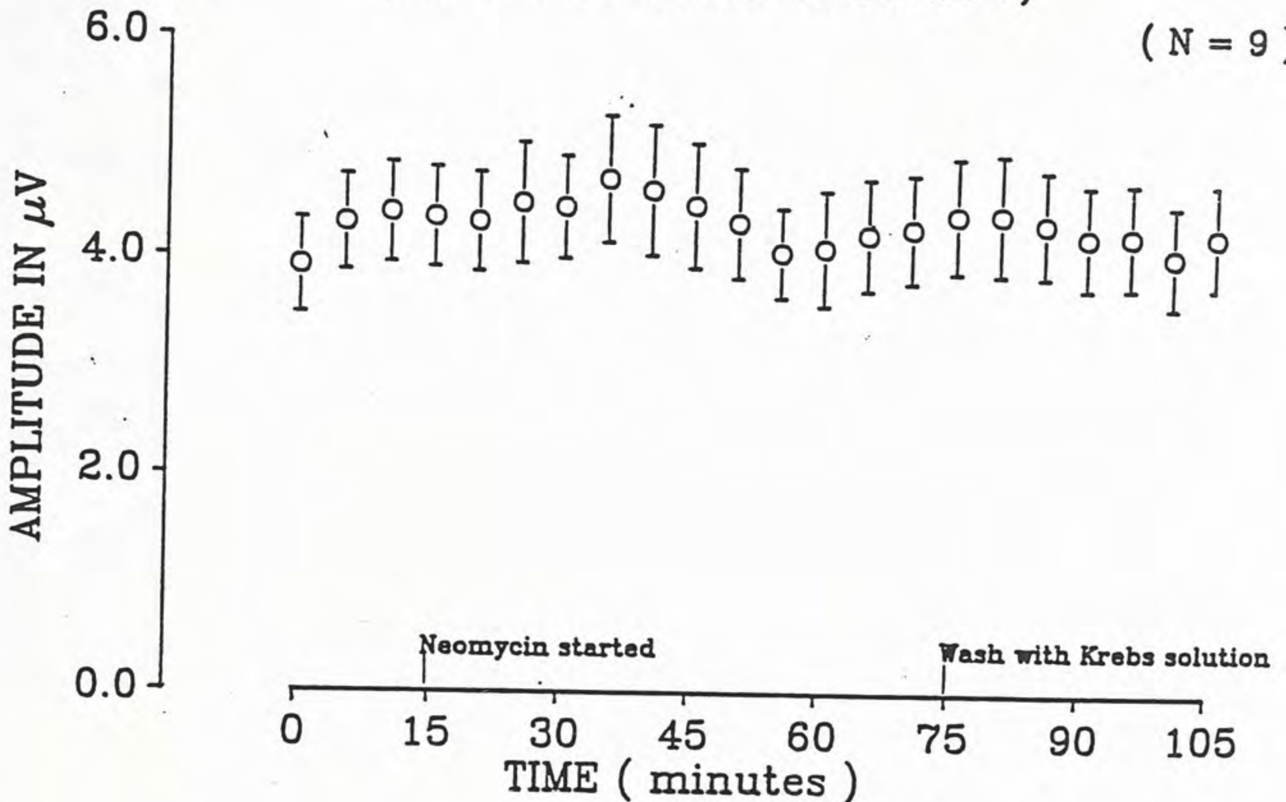


Figure 24 are the data of the C compound action potentials in 9 rat ventrolateral tail nerves. 5.7 mM neomycin solution was started from 15 minutes onward. Whole treatment period was 60 minutes. The nerve was then subsequently washed with Krebs solution. Average potential was plotted against the time. Results were expressed as mean $\pm$SEM. No significant difference from control values was found after the exposure of neomycin solution ($P > 0.05$).

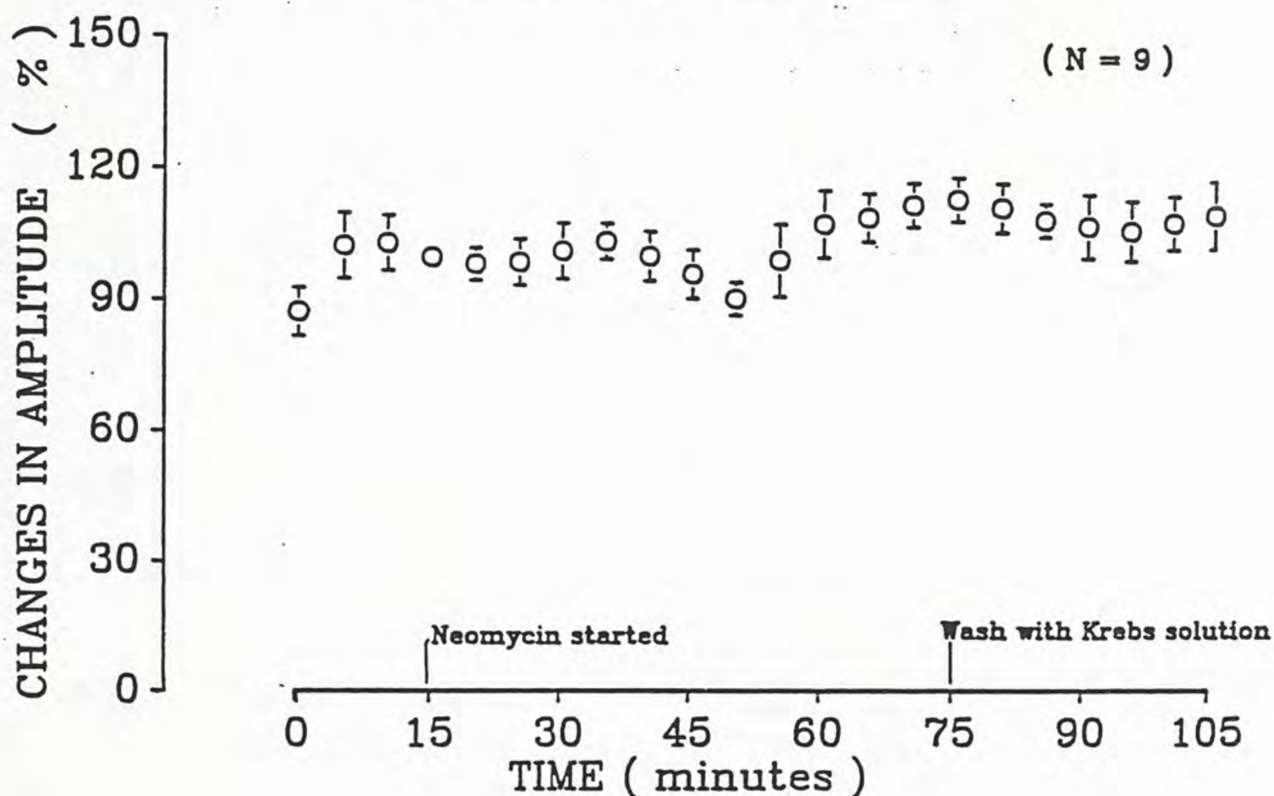


Figure 25 are the data of the C compound action potentials in 9 rat ventrolateral tail nerves. 5.7 mM neomycin solution was started from 15 minutes onward. Whole treatment period was 60 minutes. The nerve was then subsequently washed with Krebs solution. The amplitude before the application of neomycin was taken as 100%. Change in amplitude was plotted against the time. Results were expressed as mean ± SEM. No significant difference from control values was found after the exposure to neomycin solution ($P > 0.05$).

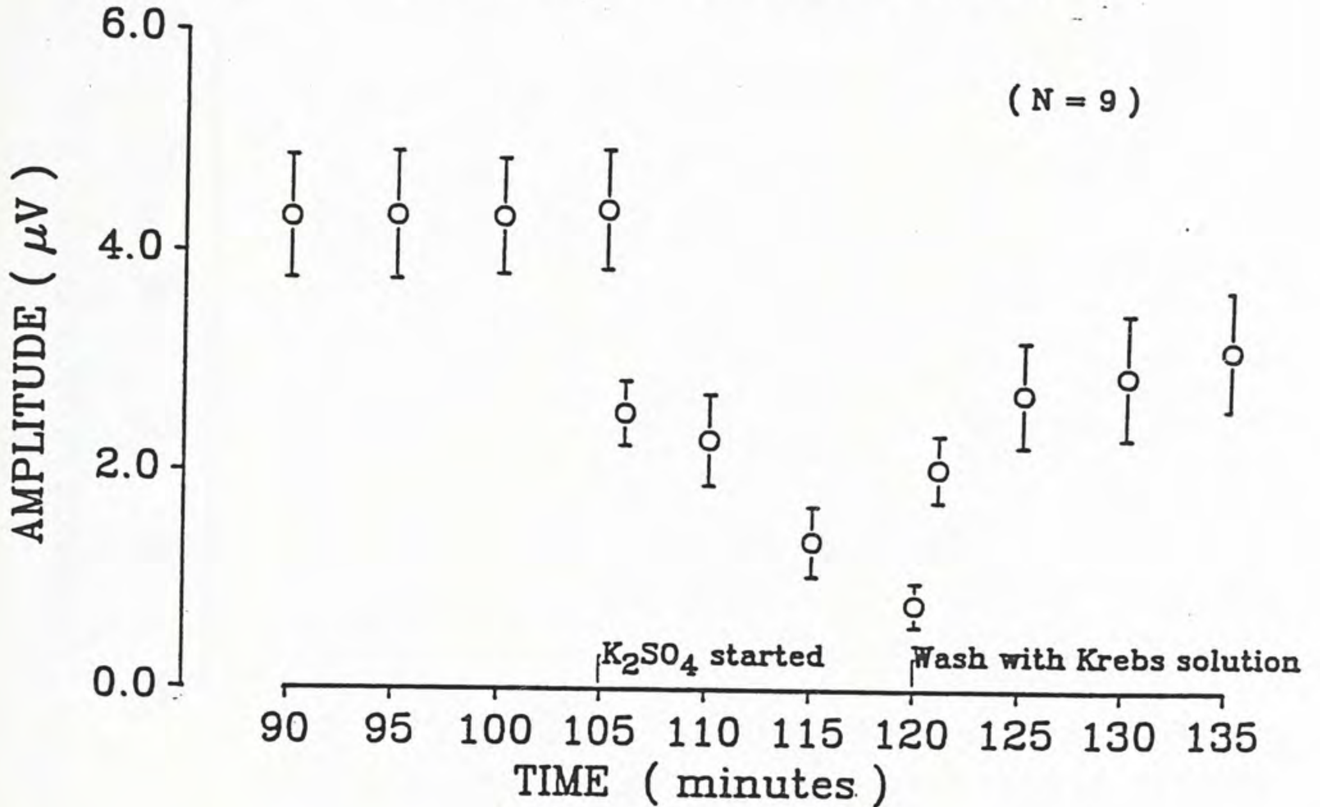


Figure 28 are the data of the C compound action potentials in 9 rat ventrolateral tail nerves treated with 10 mM K_2SO_4 Krebs solution for 15 minutes. The nerve was subsequently washed with Krebs solution. Amplitude was plotted against the time. Results were expressed as mean $\pm$ SEM. Significant difference from control values was found at 10 and 15 minutes after the exposure to 10 mM K_2SO_4 Krebs solution ($P \leq 0.05$).

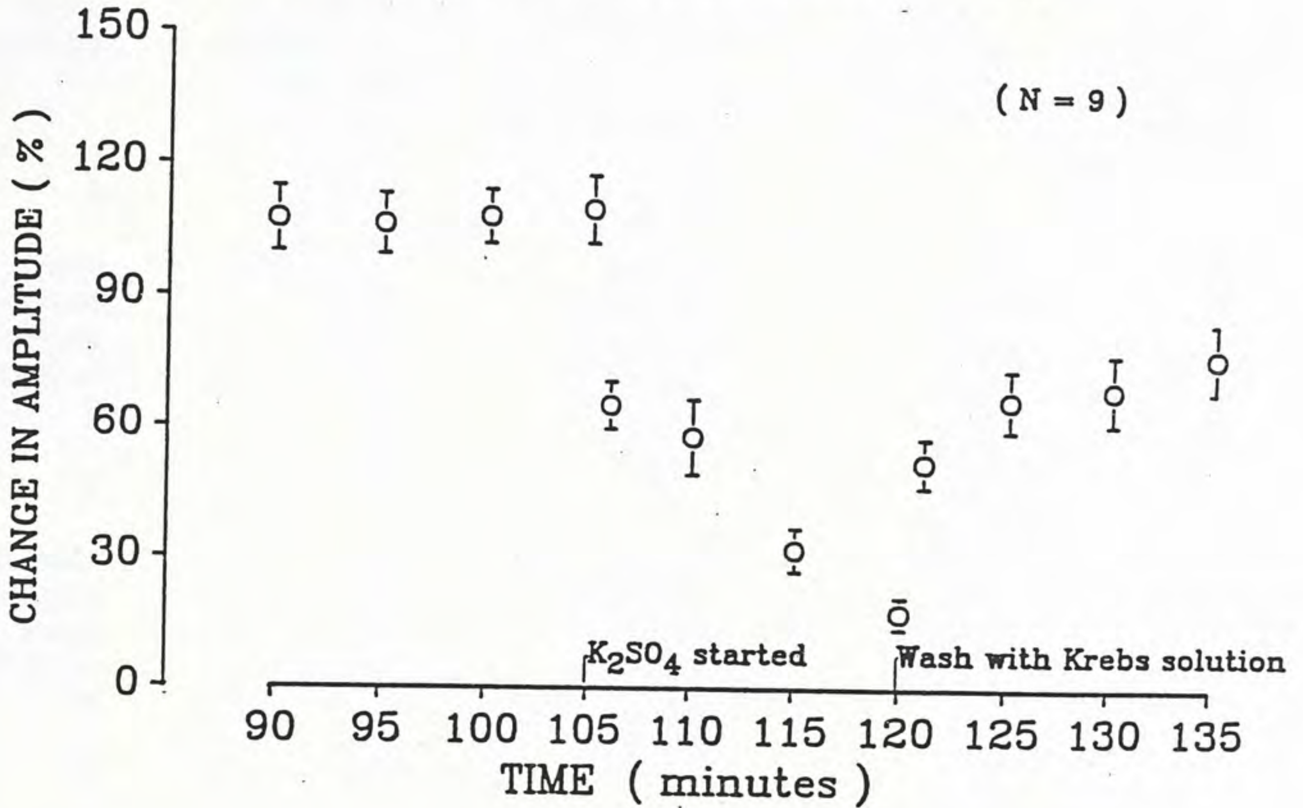


Figure 29 are the data of the A compound action potentials in 9 rat ventrolateral tail nerves treated with 10 mM K_2SO_4 Krebs solution for 15 minutes. The nerve was subsequently washed with Krebs solution. The amplitude before the application of K_2SO_4 was taken as 100%. Change in amplitude was plotted against the time. Results were expressed as mean $\pm$SEM. Significant difference from control values was found at 10 and 15 minutes after the exposure to 10 mM K_2SO_4 Krebs solution ($P \leq 0.05$).

TABLE 15

C COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF CONDUCTION VELOCITY

TIME in minutes	FASTEST C POTENTIAL in m/s	CHANGE OF CONDUCTION VELCOITY (%) OF CONTROL	SLOWEST C POTENTIAL in m/s	CHANGE OF CONDUCTION VELOCITY(%) OF CONTROL
0				
Minimum	.71	89.29	.42	79.08
Maximum	1.18	115.04	.72	132.09
Mean	.91	101.91	.55	101.04
StdDev	.16	7.81	.11	15.17
N	9	9	9	9
5				
Minimum	.71	87.27	.43	87.70
Maximum	1.12	109.88	.80	147.75
Mean	.88	98.76	.57	104.35
StdDev	.15	7.12	.12	17.35
N	9	9	9	9
10				
Minimum	.72	87.68	.47	95.19
Maximum	1.07	108.83	.81	149.19
Mean	.89	99.75	.57	105.91
StdDev	.13	7.39	.11	16.72
N	9	9	9	9
15 (Neomycin started)				
Minimum	.71	100.00	.46	100.00
Maximum	1.03	100.00	.66	100.00
Mean	.89	100.00	.54	100.00
StdDev	.12	.00	.06	.00
N	9	9	9	9
20 (5)				
Minimum	.76	81.42	.46	71.43
Maximum	1.17	114.50	.83	150.14
Mean	.91	102.46	.59	110.39
StdDev	.16	10.04	.14	25.41
N	9	9	9	9

C COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF CONDUCTION VELOCITY

TIME in minutes	FASTEST C POTENTIAL in m/s	CHANGE OF CONDUCTION VELCOITY (%) OF CONTROL	SLOWEST C POTENTIAL in m/s	CHANGE OF CONDUCTION VELOCITY(%) OF CONTROL
25 (10)				
Minimum	.76	82.68	.50	80.34
Maximum	1.20	116.16	.96	176.62
Mean	.92	103.74	.65	121.11
StdDev	.17	11.48	.15	30.02
N	9	9	9	9
30 (15)				
Minimum	.77	81.14	.49	82.61
Maximum	1.21	116.91	.86	159.51
Mean	.92	103.26	.63	117.68
StdDev	.16	12.80	.12	23.65
N	9	9	9	9
35 (20)				
Minimum	.77	81.14	.49	97.31
Maximum	1.17	141.28	.87	160.06
Mean	.95	107.98	.62	115.20
StdDev	.16	17.16	.12	17.80
N	9	9	9	9
40 (25)				
Minimum	.77	90.82	.49	96.20
Maximum	1.17	117.75	.97	186.35
Mean	.95	106.77	.65	121.67
StdDev	.16	10.57	.16	30.68
N	9	9	9	9
45 (30)				
Minimum	.79	94.94	.49	81.20
Maximum	1.23	122.97	1.10	212.71
Mean	.97	109.39	.65	122.40
StdDev	.16	9.85	.19	38.55
N	9	9	9	9

C COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF CONDUCTION VELOCITY

TIME in minutes	FASTEST C POTENTIAL in m/s	CHANGE OF CONDUCTION VELCOITY (%) OF CONTROL	SLOWEST C POTENTIAL in m/s	CHANGE OF CONDUCTION VELOCITY(%) OF CONTROL
50 (35)				
Minimum	.81	93.58	.51	80.85
Maximum	1.14	119.20	.81	150.16
Mean	.94	106.49	.60	111.95
StdDev	.12	9.10	.11	22.81
N	9	9	9	9
55 (40)				
Minimum	.76	90.63	.53	81.55
Maximum	1.10	123.78	.77	141.41
Mean	.92	104.25	.58	108.98
StdDev	.12	11.18	.08	16.87
N	9	9	9	9
60 (45)				
Minimum	.85	91.13	.50	93.60
Maximum	1.12	128.27	1.05	192.88
Mean	.96	108.51	.66	123.10
StdDev	.10	12.22	.16	30.75
N	9	9	9	9
65 (50)				
Minimum	.81	86.42	.54	86.17
Maximum	1.24	127.35	.92	169.48
Mean	.96	108.83	.65	120.72
StdDev	.14	13.11	.12	24.80
N	9	9	9	9
70 (55)				
Minimum	.78	83.71	.53	84.26
Maximum	1.42	137.50	.95	175.28
Mean	.97	109.23	.66	123.18
StdDev	.19	16.66	.14	27.44
N	9	9	9	9

C COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF CONDUCTION VELOCITY

TIME in minutes	FASTEST C POTENTIAL in m/s	CHANGE OF CONDUCTION VELCOITY (%) OF CONTROL	SLOWEST C POTENTIAL in m/s	CHANGE OF CONDUCTION VELOCITY(%) OF CONTROL
75 (60) "wash with Krebs solution"				
Minimum	.75	80.44	.52	78.67
Maximum	1.48	143.19	.97	177.98
Mean	.96	108.27	.64	118.81
StdDev	.22	17.69	.14	27.47
N	9	9	9	9
80 "5"				
Minimum	.80	85.59	.49	74.22
Maximum	1.48	143.19	.95	175.95
Mean	.96	108.33	.61	115.08
StdDev	.21	15.98	.14	27.56
N	9	9	9	9
85 "10"				
Minimum	.82	87.55	.43	66.09
Maximum	1.49	144.04	.91	167.63
Mean	.98	110.19	.60	112.81
StdDev	.20	17.63	.15	29.05
N	9	9	9	9
90 "15"				
Minimum	.85	83.60	.52	78.51
Maximum	1.36	131.52	.90	165.23
Mean	.96	109.32	.64	119.58
StdDev	.15	16.77	.12	24.45
N	9	9	9	9
95 "20"				
Minimum	.86	90.87	.49	74.95
Maximum	1.29	127.81	.85	158.84
Mean	.96	108.54	.65	122.11
StdDev	.13	12.96	.12	25.84
N	9	9	9	9

C COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF CONDUCTION VELOCITY

TIME in minutes	FASTEST C POTENTIAL in m/s	CHANGE OF CONDUCTION VELCOITY (%) OF CONTROL	SLOWEST C POTENTIAL in m/s	CHANGE OF CONDUCTION VELOCITY(%) OF CONTROL
100 "25"				
Minimum	.84	94.96	.57	87.36
Maximum	1.28	126.44	1.02	187.39
Mean	.97	109.52	.68	128.10
StdDev	.13	10.43	.14	29.66
N	9	9	9	9
105 "30" '10 mM K_2SO_4 started'				
Minimum	.78	93.51	.56	86.17
Maximum	1.18	124.66	1.05	192.88
Mean	.95	107.30	.65	120.95
StdDev	.13	9.36	.15	29.77
N	9	9	9	9
106 '1'				
Minimum	.34	33.29	0.00	0.00
Maximum	1.24	121.31	.69	119.18
Mean	.83	94.56	.47	89.47
StdDev	.24	28.05	.19	35.93
N	9	9	9	9
110 '5'				
Minimum	.34	33.06	.34	60.61
Maximum	.85	98.81	.60	105.61
Mean	.64	73.18	.46	85.99
StdDev	.18	22.57	.09	17.87
N	9	9	9	9
115 '10'				
Minimum	.34	33.06	.34	53.45
Maximum	1.06	103.82	.51	98.03
Mean	.57	64.04	.41	76.38
StdDev	.24	23.57	.07	17.39
N	9	9	9	9

C COMPOUND ACTION POTENTIAL
RAT VENTROLATERAL TAIL NERVE
DATA OF CONDUCTION VELOCITY

TIME in minutes	FASTEST C POTENTIAL in m/s	CHANGE OF CONDUCTION VELCOITY (%) OF CONTROL	SLOWEST C POTENTIAL in m/s	CHANGE OF CONDUCTION VELOCITY(%) OF CONTROL
120	'15' (wash with Krebs solution)			
Minimum	0.00	0.00	0.00	0.00
Maximum	.74	72.04	.49	89.69
Mean	.45	49.04	.36	65.59
StdDev	.22	23.66	.15	28.86
N	9	9	9	9
121	(1)			
Minimum	.50	48.40	.43	86.77
Maximum	1.08	124.82	1.17	204.25
Mean	.82	93.70	.63	116.06
StdDev	.17	23.67	.22	35.86
N	9	9	9	9
125	(5)			
Minimum	.53	51.06	.46	82.25
Maximum	1.15	131.75	.76	140.97
Mean	.82	94.10	.58	107.25
StdDev	.18	25.31	.10	17.82
N	9	9	9	9
130	(10)			
Minimum	.55	53.31	.50	78.67
Maximum	1.27	142.50	.86	157.87
Mean	.87	99.99	.60	112.80
StdDev	.22	27.53	.11	23.24
N	9	9	9	9
135	(15)			
Minimum	.56	54.51	.44	66.43
Maximum	1.27	146.26	.78	143.16
Mean	.89	101.63	.60	111.91
StdDev	.22	28.84	.10	23.02

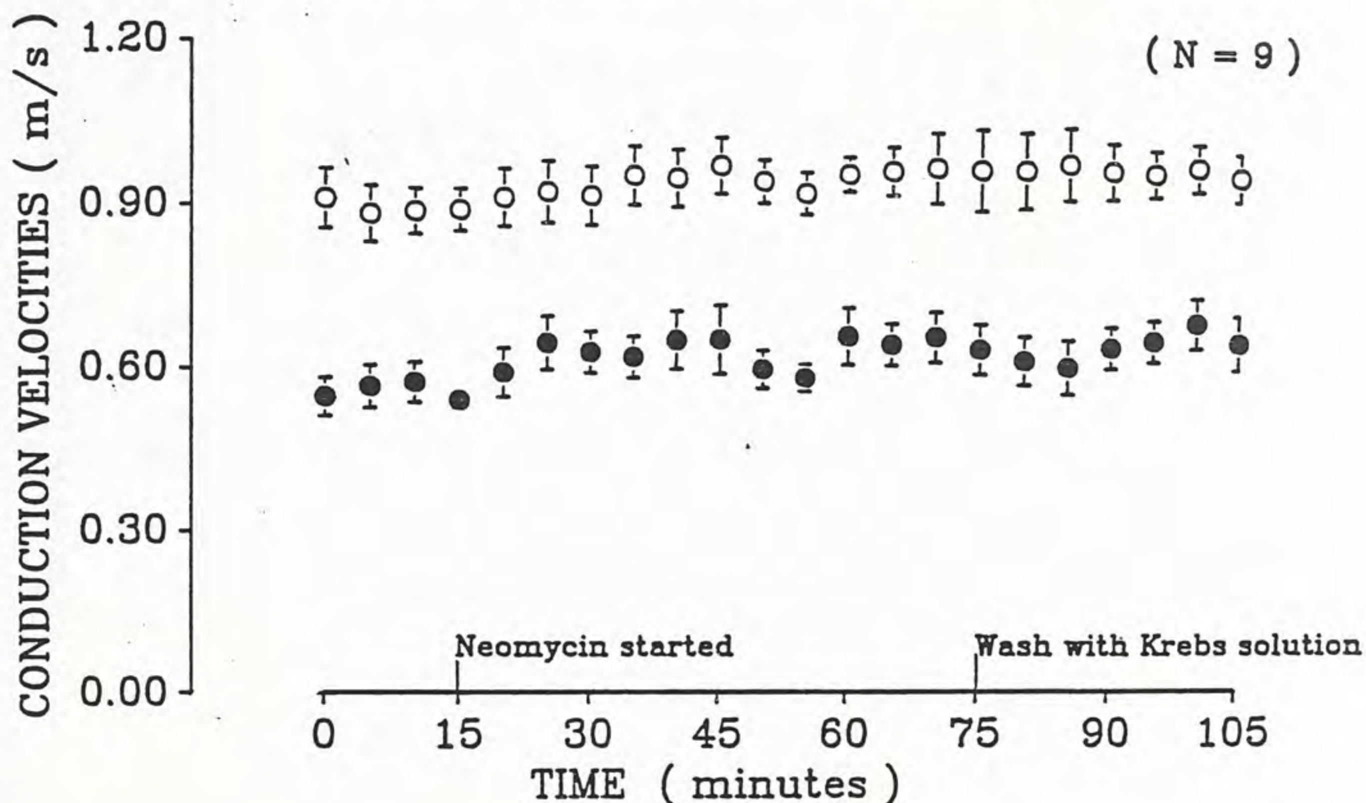


Figure 26 are the data of the C compound action potentials in 9 rat ventrolateral tail nerves. 5.7 mM neomycin solution was started from 15 minutes onward. Whole treatment period was 60 minutes. The nerve was then subsequently washed with Krebs solution. The fastest and slowest C potential were recorded (0 and 0 respectively). Conduction velocities were plotted against the time. Results were expressed as mean $\pm$ SEM. No significant difference from control values was found after the exposure to neomycin solution ($P > 0.05$).

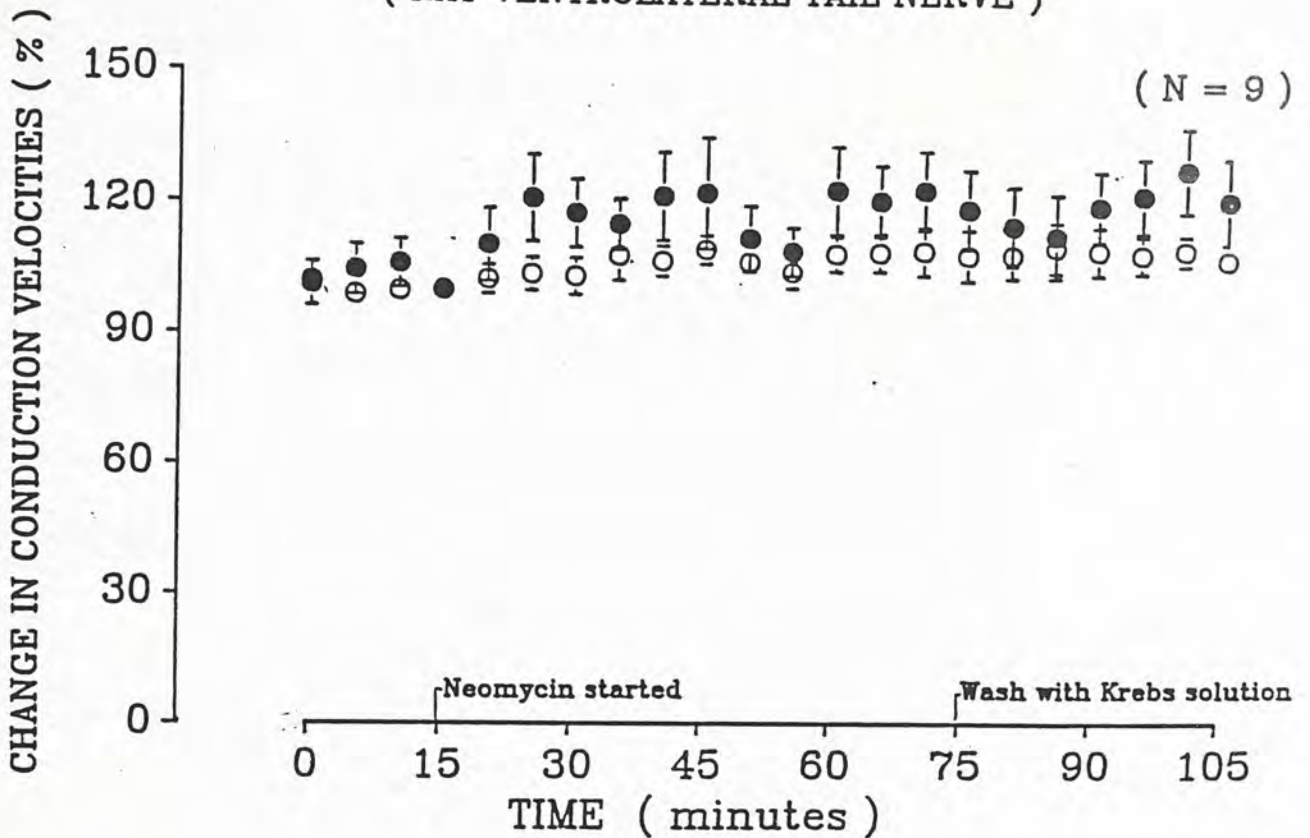


Figure 27 are the data of the C compound action potentials in 9 rat ventrolateral tail nerves. 5.7 mM neomycin solution was started from 15 minutes onward. Whole treatment period was 60 minutes. The nerve was then subsequently washed with Krebs solution. The fastest and slowest C potential were recorded (0 and 0 respectively). The conduction velocities before the application of neomycin solution were taken as 100 %. Changes in conduction velocity were plotted against the time. Results were expressed as mean $\pm$SEM. No significant difference from control values was found after the exposure to neomycin solution ($P > 0.05$).

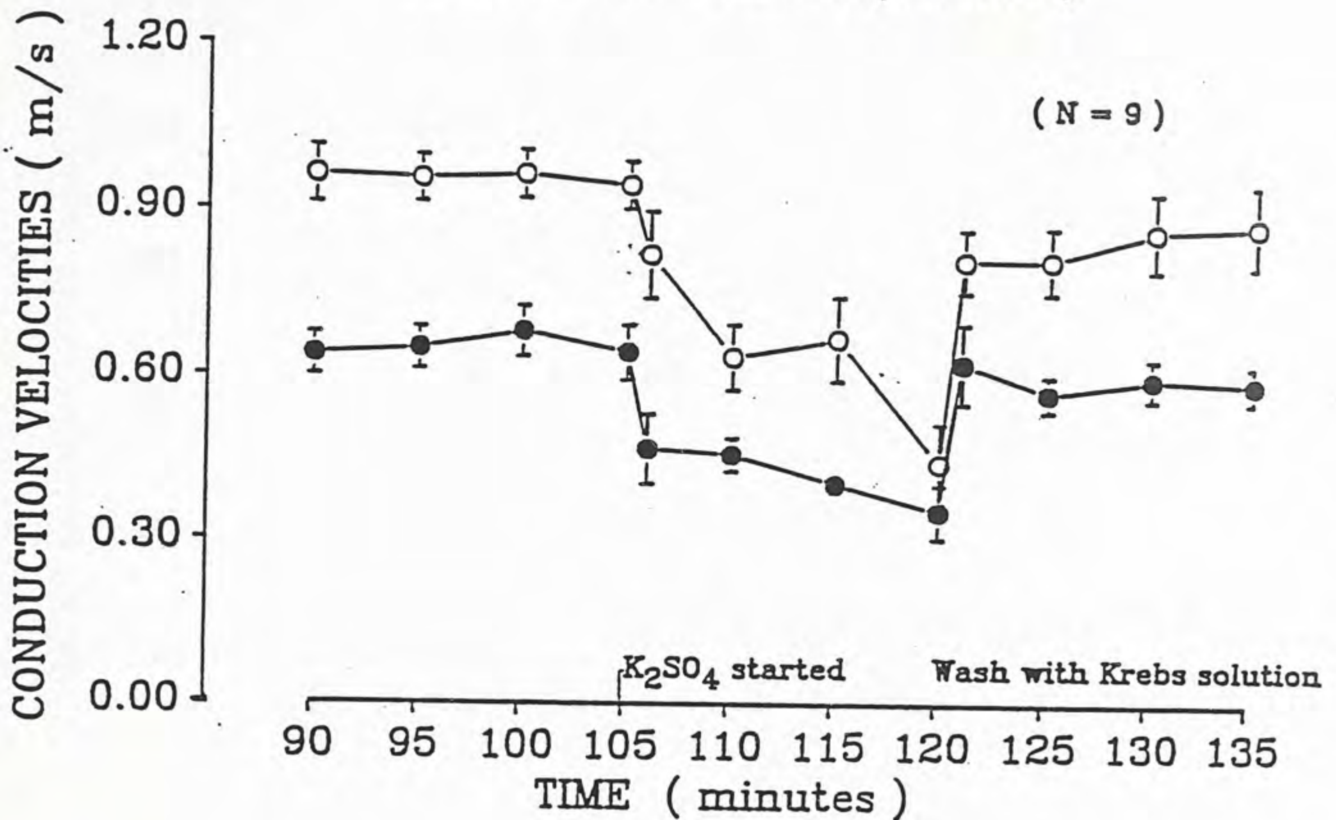


Figure 30 were the data of the C compound action potentials in 9 rat ventrolateral tail nerves treated with 10 mM K_2SO_4 Krebs solution for 15 minutes. The nerve was subsequently washed with Krebs solution. The fastest and slowest C potential were recorded (0 and ● respectively). Conduction velocities were plotted against the time. Results were expressed as mean $\pm$SEM. Significant difference from control values was found at 10 and 15 minutes after the exposure to 10 mM K_2SO_4 Krebs solution ($P \leq 0.05$).

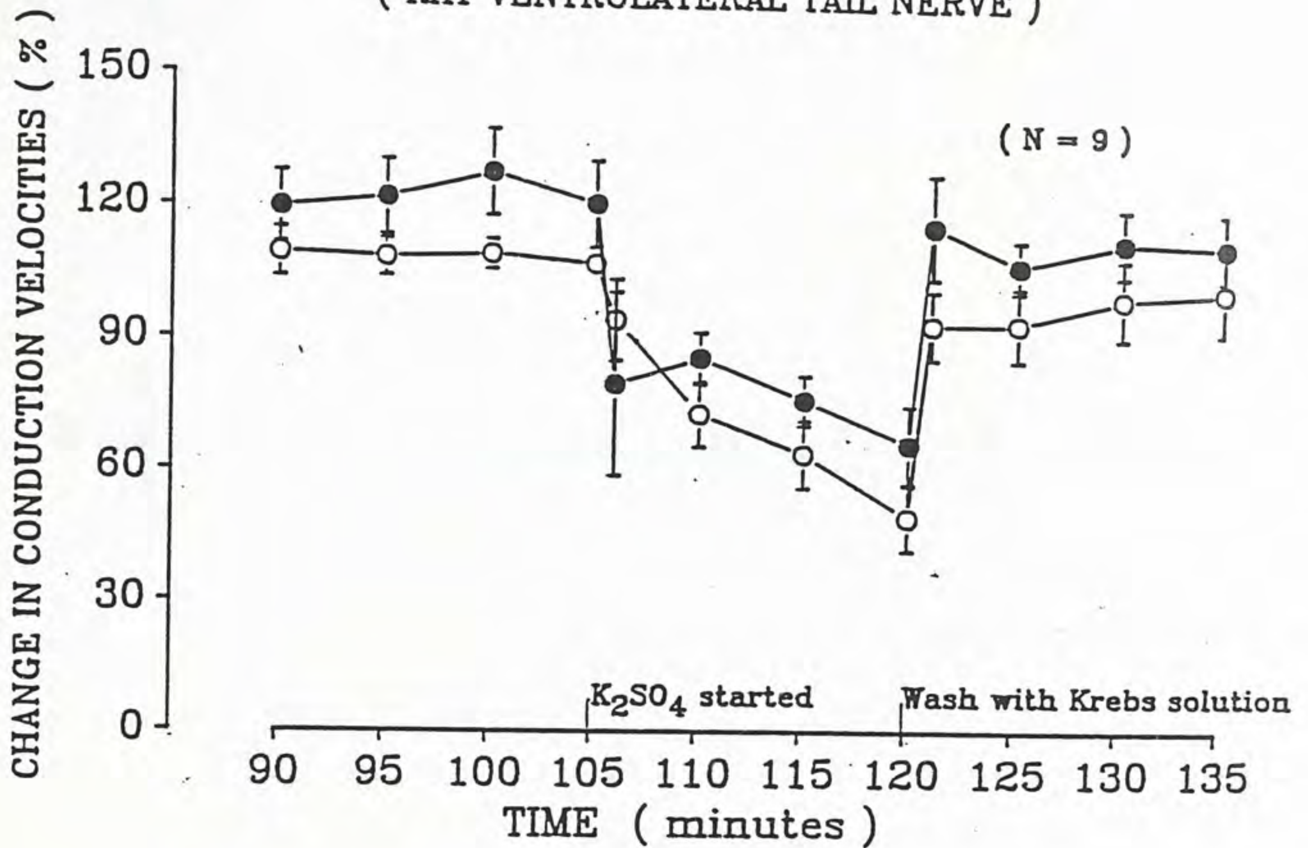


Figure 31 are the data of the C compound action potentials in 9 rat ventrolateral tail nerves treated with 10 mM K_2SO_4 Krebs solution for 15 minutes. The nerve was subsequently washed with Krebs solution. The fastest and slowest C potential were recorded (0 and ● respectively). The conduction velocities before the application of K_2SO_4 solution were taken as 100 %. Change in conduction velocities were plotted against the time. Results were expressed as mean $\pm$SEM. Significant difference from control values was found at 10 and 15 minutes after the exposure to 10 mM K_2SO_4 Krebs solution ($P \leq 0.05$).

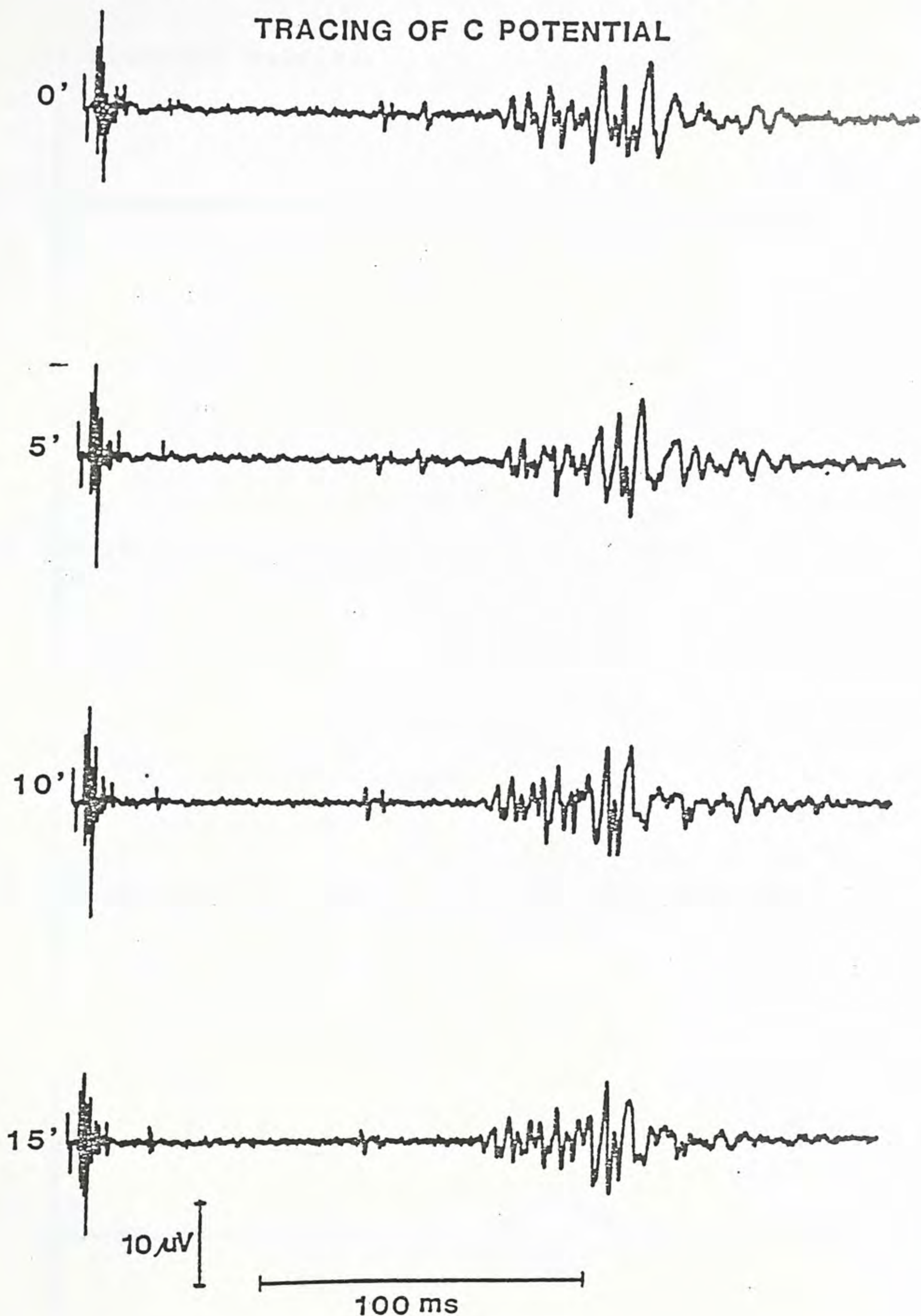
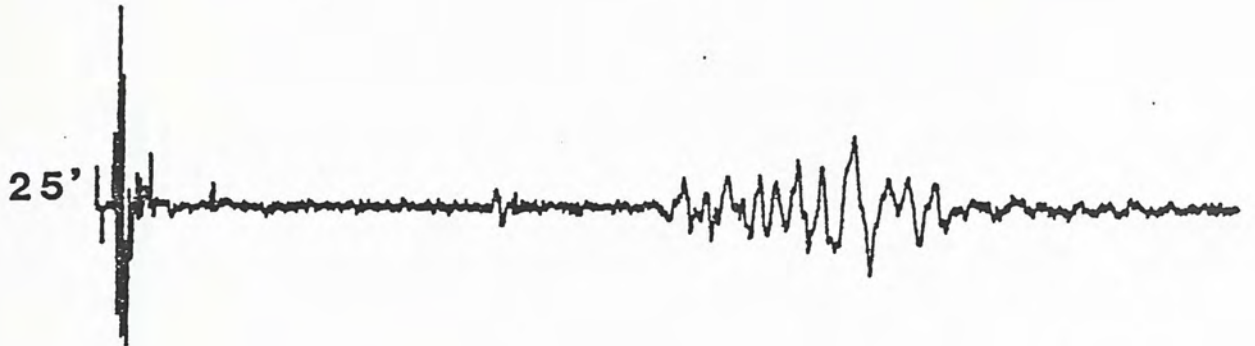
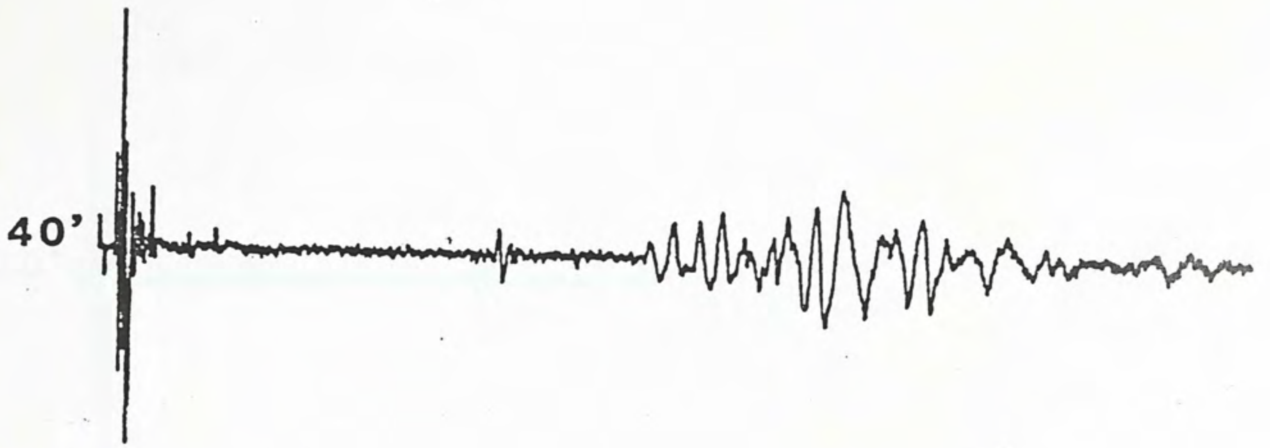


Figure 32 In vivo study of C compound action potential in rat ventrolateral tail nerve, original recording of the action potential with exposure on 5.7 mM neomycin solution in Krebs solution for 60 minutes, washed with Krebs solution for 30 minutes and subsequent exposure to 10 mM K_2SO_4 to shown the blockade effect. Conductive distance was 9.7 cm.

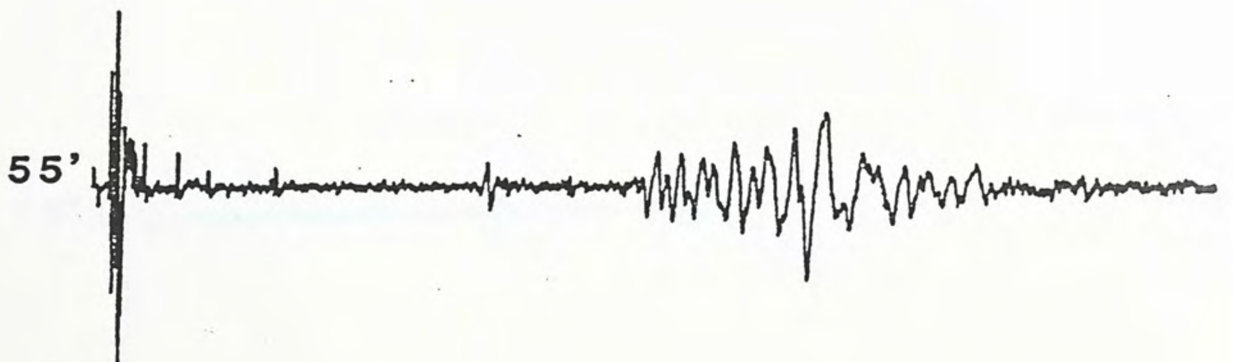
Neomycin started

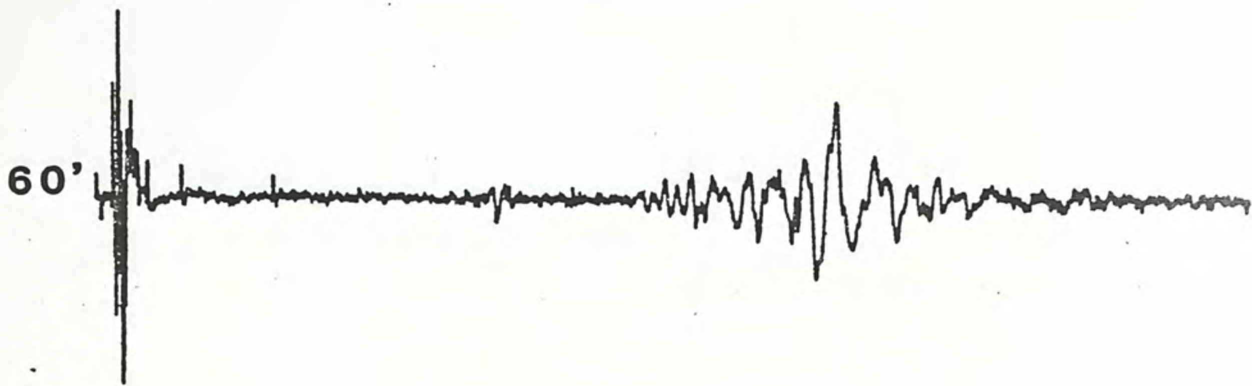



40'

45'

50'


55'


60'

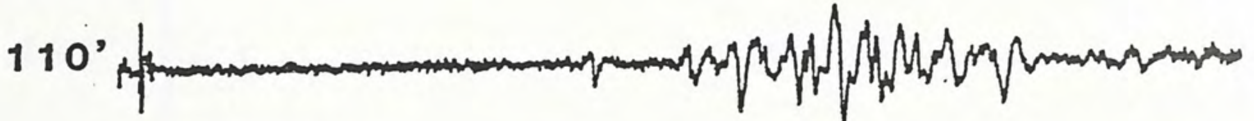
65'

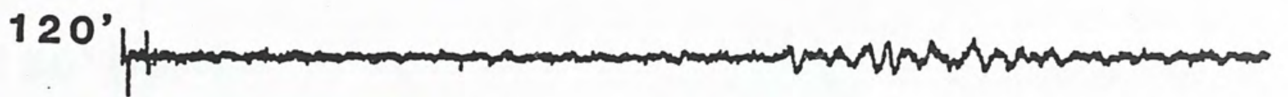
70'

75'

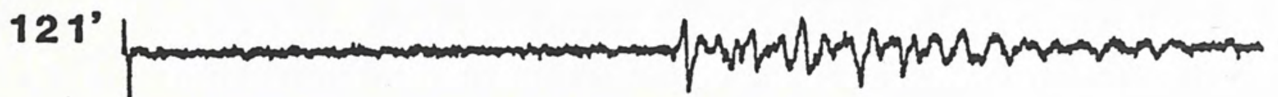
Wash with Krebs solution

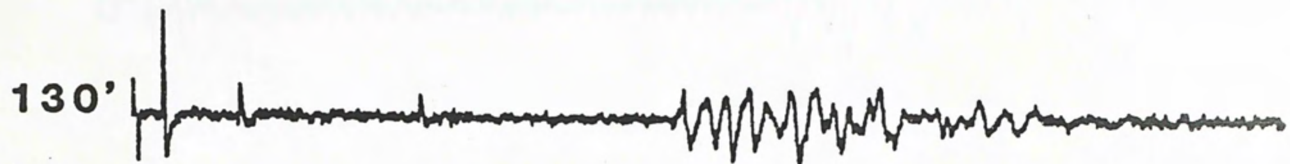
10mM K_2SO_4 started





wash with Krebs solution




130'

135'

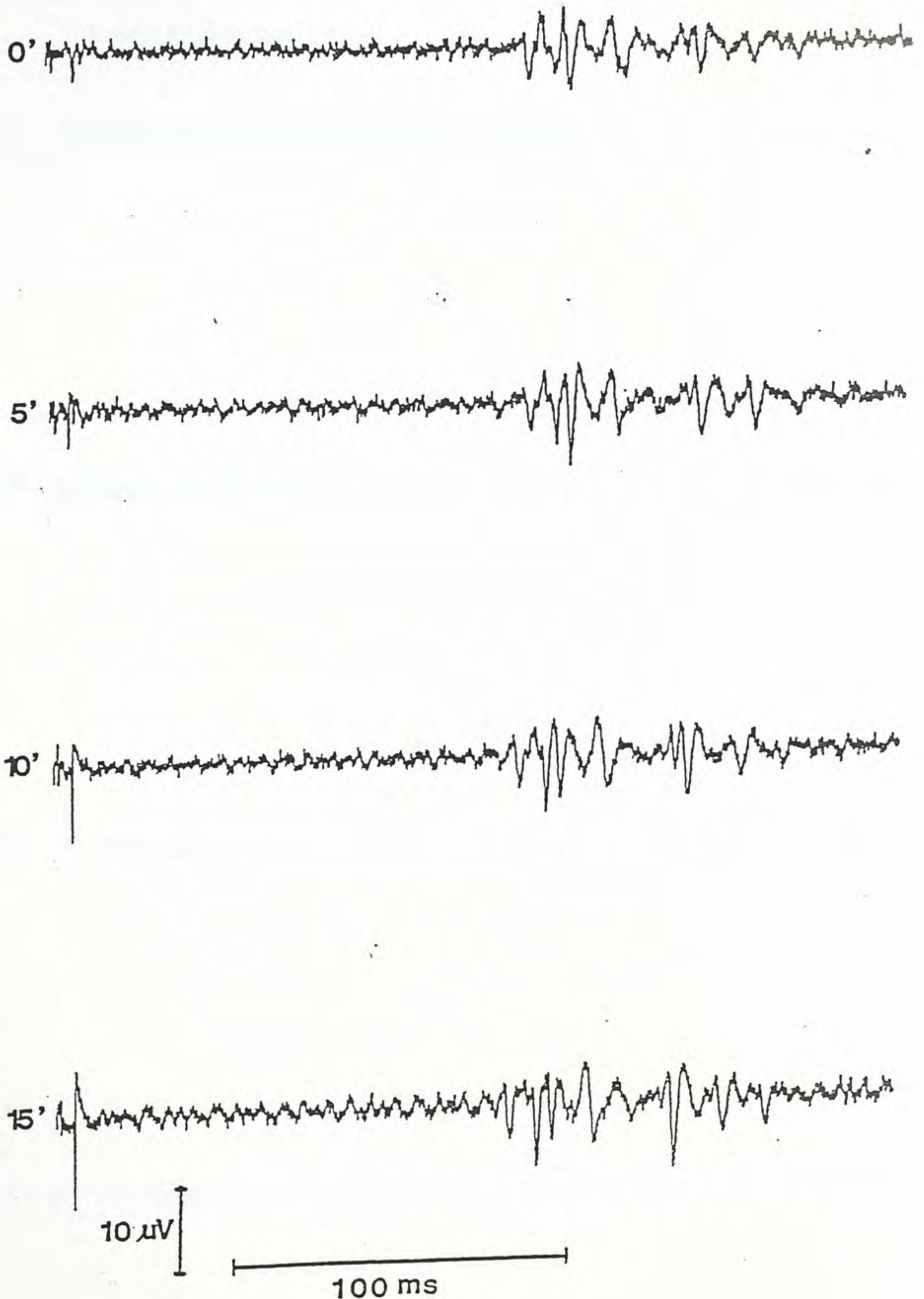
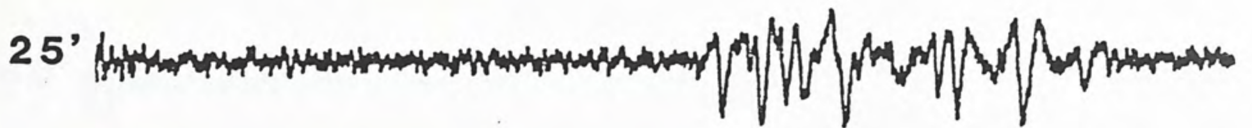


Figure 33 In vivo study of C compound action potential in rat ventrolateral tail nerve, original recording of the action potential with exposure on 5.7 mM neomycin solution in Krebs solution for 60 minutes, washed with Krebs solution for 30 minutes and subsequent exposure to 10 mM K_2SO_4 to shown the blockade effect. Conductive distance was 10.8 cm.

Neomycin started



40'

45'

50'

55'

60'

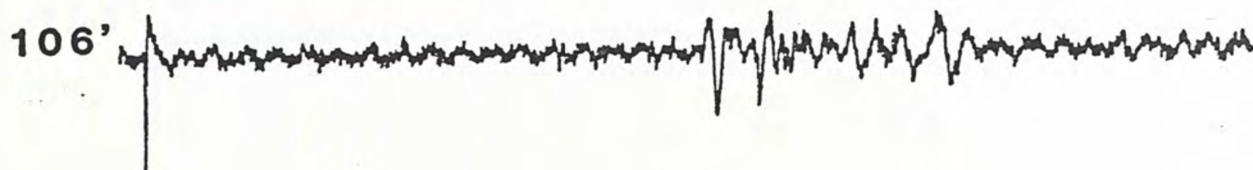
65'

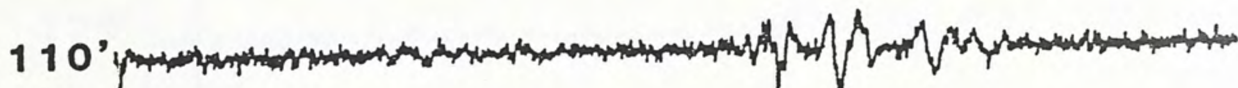
70'

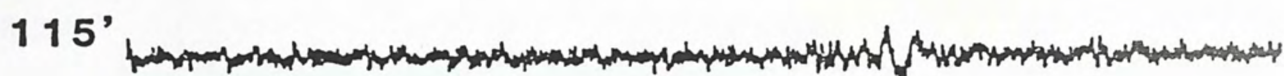
75'

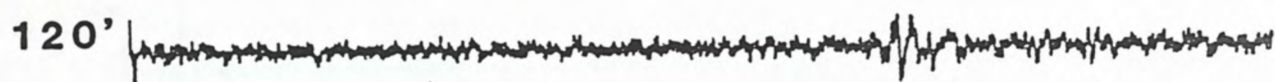
Wash with Krebs solution

10 mM K_2SO_4 started

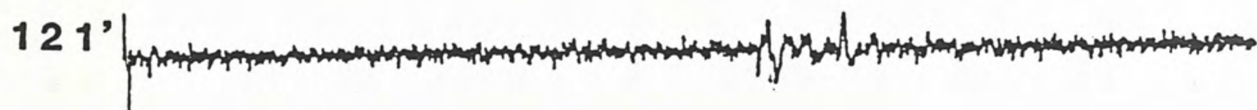


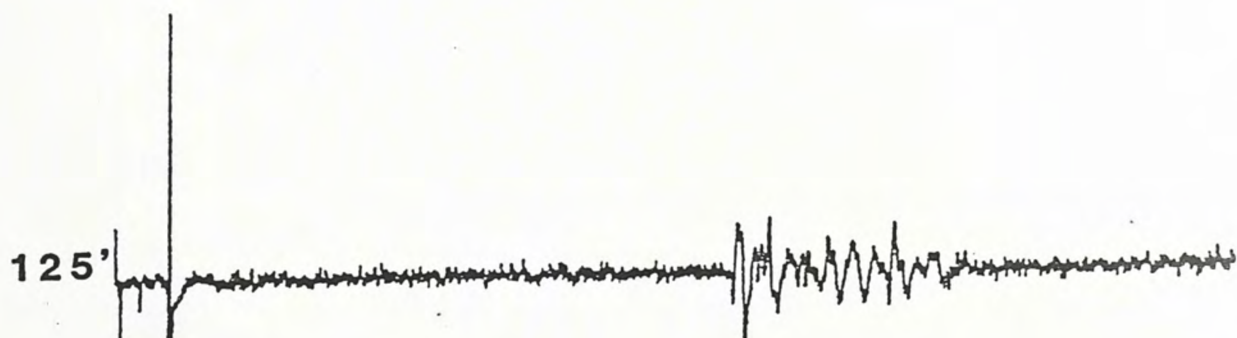


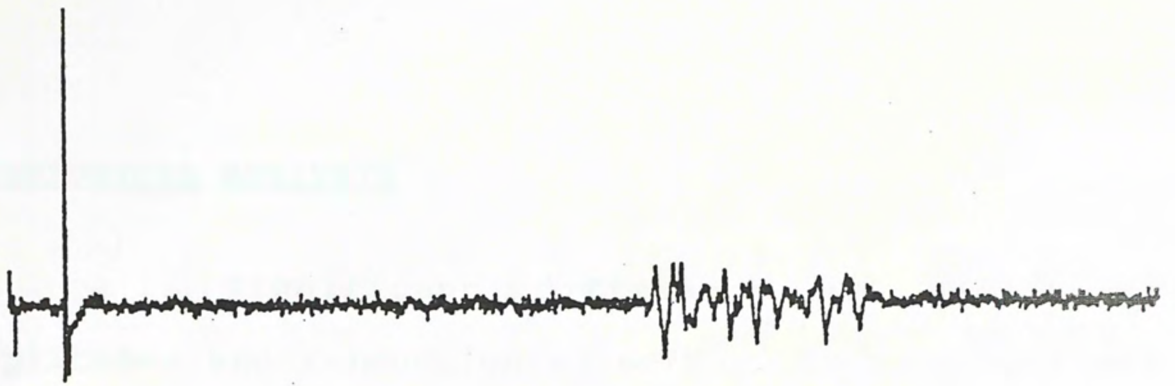


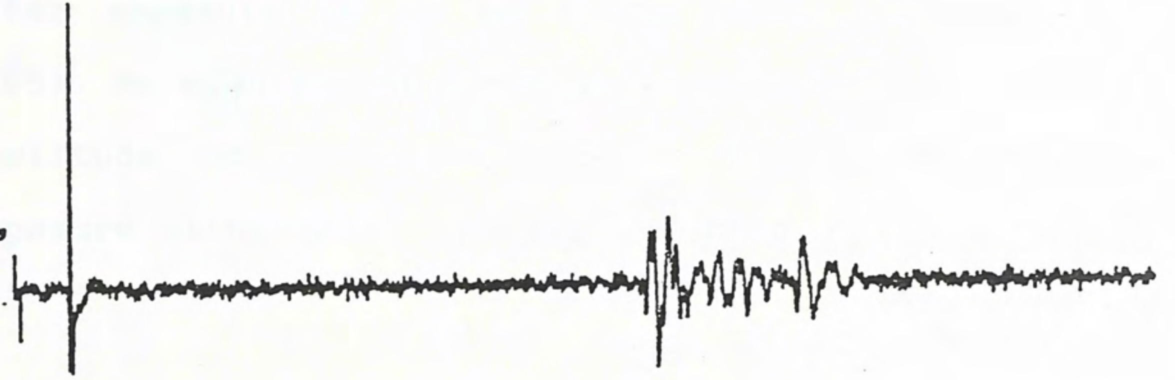


Wash with Krebs solution






130'


135'

STATISTICAL ANALYSIS

Significant difference in change of amplitudes and conduction velocities from control was found at 10, 15 minutes and 15 minutes respectively after expsoure to 10 mM K_2SO_4 Krebs solution ($p < 0.05$). No significant difference was found in change in amplitude and conduction velocity before and after the exposure of neomycin in Krebs solution.

SECTION V:- DISCUSSION

SECTION V:-DISCUSSION

Since the isolation of neomycin from Streptomyces fradiae by Waksman and Lechevalier in 1949, neomycin has been used successfully in treating infection caused by gram-negative bacteria. Beside the therapeutic significance, the aminoglycoside is also well known of for ototoxicity, nephrotoxicity, neuromuscular blockade effect and modulation of the normal function of several excitable tissues (Schtbeci and Schacht, 1977; Schacht, 1986; Hawkins, 1976; Baumann, Hamann and Leung,1988). Recently, streptomycin has been used successfully in the treatment of idiopathic trigeminal neuralgia (Sokolovic, Todorovic, Stajeic and Petrovic 1986). From the works of the above authors, the aminoglycoside may act diversely on cellular mechanisms. The aim of the present study is to investigate the possible effects of neomycin on the conduction in nerve fibres. Transmission of information or signal carrying in nerve fibres is effected through the propagation of action potentials.

The action potential serves to conduct an electrical impulse with undiminished strength along the length of the nerve fibre. Propagation of the action potential occurs by the mechanism of depolarization and re-polarization. When the areas on either side of the depolarized region reach the threshold, an action potential will be generated. By local current flows, the

areas adjacent to these will be brought to threshold and will in turn generate an action potential. Through this process, action potentials propagate over the whole length of nerve fibres in the same shape and size. There are four clearly defined phases in an action potential. The rising phase (in which the membrane potential with respect to the extracellular space becomes more positive), the peak, the falling phase (in which the membrane potential returns to its original negativity) and the "underswing" phase (in which the membrane potential is actually more negative than under resting conditions). The duration of the action potential in nerve fibres is only a few milliseconds.

In the preliminary series of experiment on frog, sciatic nerve was used. It was because the nerve itself was relatively easy to dissect, and the results can be compared with those from other workers like Sokoll and Diecke (1969) who had carried out similar experiments with streptomycin.

The original hypothesis was that acute exposure to neomycin may have an acute effect on the nerve fibre. Frequent stimulation and monitoring of the nervous impulses was necessary. Therefore, stimulation was given every minute for 30 minutes. The preliminary series of the experiment did not produce any positive result. A longer duration of exposure may be necessary before neomycin can exert its effect on the nerve. Exposure of

the nerve for 1 hour was performed on the rat ventrolateral tail nerve. Stimulation was given every 5 minutes.

Acute exposure of myelineated nerve fibres to neomycin with concentration of 350 mg/l to 1400 mg/l on the rabbit sural nerve had been studied by Chan et al. (1985). There was no apparent acute functional impairment of nervous conduction. A higher concentration may be necessary to reveal the effects of neomycin on the nerve fibres. A concentration of 3500 mg/l was thus used in the present experiment.

1.1 CONDUCTION VELOCITIES OF FROG SCIATIC NERVE AND RAT-TAIL NERVE

In the studies of generation and propagation of nervous impulse in nerve fibres, amplitudes and the conduction velocities of the action potential are two parameters that have to be measured. The measurement of conduction velocity is subject to some degree of uncertainty. As a measure of speed, conduction velocity is derived from the estimation of time and distance. Hence factors affecting either temporal or spatial resolution will contribute to the uncertainty of the conduction velocity measurement. The quantifiable factors include the minimum sampling time of the averaging technique and the background noise. Both of these will affect conduction latency resolution. The

finite resolution and subjective placement of the caliper between the stimulating and recording electrode would contribute to the error in the measurement of conductive distance (Gordon, Hamm, Enoka, Reinking, Windhorst and Stuart, 1987).

It has been well established that the conduction velocity of the nervous impulses decreases with the fall of the body / surrounding temperature (Hodgkin and Katz 1949 ; Paintal 1965). The conduction velocity in amphibians e.g. the frog will be far slower than in mammalian species. The mean conduction velocity of the A compound action potential of frog sciatic nerve obtained in here are comparable with other workers like Meyer and Hegmann (1971).

The rat tail occupies about 12.8% of the surface area for a rat weight 350g (Lyzak and Hunter, 1987). It is the main site of heat dissipation during body heating. Heat dissipation through the tail occurs largely through increase in rat tail blood flow and vasodilation in the rat-tail accompanying heating occurs solely via reduction in sympathetic vasoconstrictor nerve activity (O'Leary, Johnson and Taylor, 1985). The rat tail temperature in resting state is far below the body core temperature. Therefore, maintenance of body temperature of the rat tail is important to get a comparable conduction velocity in the nervous impulse.

It is achieved by maintaining the core temperature of the rat by putting the rat on a thermostatically controlled electric blanket (Bioscience CFP-8185). To maintain the rat tail temperature, bathing the rat tail in paraffin oil had been used by some workers (Miyoshi and Goto, 1973; Ono, Takeuchi and Hisanaga, 1979). This method only allows transcutaneous stimulation of the nerve fibres. The present experiments make use of a U-shaped glass tube to embed the rat tail allows the maintenance of the rat tail temperature as well as direct stimulation and recording of the nerve fibres.

Birren and Wall (1956) reported that motor conduction velocities increase at a rate of about 1.2 m/s per degree between 20 ^{o}C and 40 ^{o}C in vitro experiments on rat sciatic nerve. Miyoshi and Goto (1973) gave more direct measurements of the rat-tail nerve. They estimated that the conduction velocity of rat-tail increases linearly at a rate of about 1.3 m/s per degree between 24 ^{o}C and 42 ^{o}C. They also stated that the conduction velocity increases during growth to about 300 days. In in vitro experiments, they found that when the mean subcutaneous temperature was 36.4 $\pm$0.7 C, the mean conduction velocity of the A potential in Sprague-Dawley rat was 34.8 $\pm$3.2 m/s. in 100 day old rats. Taken into consideration of the mean temperature in the present studies, the corresponding conduction velocity should be about 30 m/s. One factor that may

account for the slower velocities may be the damage of the capillaries which supply the nerve fibres. As a result, the vascular supplies which maintain the temperature of the nerve fibre is disturbed. In addition, the temperature recorded subcutaneously may not actually correspond to the temperature at the recording site. Taken all these points into consideration, the results obtained here correspond well with the studies of Miyoshi and Goto (1973) and Ono and his coworkers (1979).

Conduction velocities of the fastest and slowest C potential in the whole spectrum of C potential were selected in the present experiment. In the present experiment, conductive distance of about 8-10 cm. was used frequently. The amplitudes of the C potentials showed several volleys which were dispersed quite widely. The latent period was from the range of 100 to 200 ms for a conductive distance of 8-10 cm. The conduction velocities of the C potential recorded here are well below 1.5 m/s which agrees well with classification by Gasser (Gasser, 1950).

1.2 AMPLITUDES OF THE ACTION POTENTIAL

The amplitude of the compound action potential depends on a number of factors including the distance between the neural membrane (axolemma) and the recording electrodes, the distance between the two electrodes and

the electrical properties of the interposing, non-neural tissue. The amplitudes diminish with increasing distance from the axis of the nerve. This radial dependence was of exponential decay with increasing distance from the nerve (Plonsy and Fleming 1969). The shape of the compound action potential will also be affected by the diversity of conduction velocities in different nerve fibres. This will be more apparent when the conduction distance decreased.

In the studies of the compound action potential of the whole nerve or strands of nerve fibres, the whole spectrum of the A potential falls within the same peak in the frog sciatic nerve. Peak potential is selected to represent the amplitude of the whole A potential. In the rat ventrolateral tail nerve, the A and C potential spread out widely. There will be drawback if selection of peak potentials are used to represent the whole range of the action potential. The decrease in one peak may be compensated by increase in amplitude in other peak potentials which had not been monitored. As a result of this, the whole algebraic sum of the potentials may be the same but the selected peak potential may be changed tremendously. The data analysis program developed for the present experiment help to solve this problem. The whole range of the A or C potential was first located on screen by the cursor which gave an accuracy of 0.1 ms. Full wave rectification was performed. Integration of

the range divided by the interval gave the average amplitude of A and C potential respectively. The program also provided automatic determination of the baseline or zero line level which was done by sampling the ground noise level before the stimulation.

1.3 EFFECTS OF NEOMYCIN ON THE NERVE FIBRES

It has been well documentated that the toxicities of the aminoglycosides include neuromuscular blockade (Adams and Matthew, 1973; Singh, Harvey and Marshall, 1978; Rosayro and Healy, 1978; Fickers, 1983), nephrotoxicity (Schtbeci and Schact, 1977; Hagiwara et al., 1988), ototoxicity (Hawkins, 1976) and reduction of responsiveness in touch corpuscles (Baumann, Hamann and Leung, 1986). As far as the neuromuscular blockade is concerned, the basic elements of normal neuromuscular transmission that can be affected by antibiotics are 1. action potential transmission in the motor nerve and motor nerve terminal; 2. the process of synthesis, mobilization and release of acetylcholine from the nerve terminal; 3. the integrity of the post-synaptic cholinergic receptor; 4. action potential generation and propagation in the muscle membrane; 5. normal excitation-contraction coupling in the muscles.

The neuromuscular blockade by neomycin was associated with a large significant decrease in evoked release of acetylcholine and with a smaller but still

significant decrease in post-junctional receptor sensitivity and spontaneous release (Adams and Matthew, 1973; Wright and Collier, 1977). The reduced transmitter release is by a mechanism similar to that of magnesium ions which involves a competition for calcium ions binding sites on the nerve terminal (Prado et al., 1978).

Little knowledge is available about the effect of neomycin on the generation and propagation of the action potential in the nerve fibers. From the pathological findings due to aminoglycoside ototoxicity in man, it was concluded that the nerve fibres become affected only when the hair cells were missing. Nerve fibre degeneration was secondary to hair cell degeneration. The distal processes of the first order neurons tend to degenerate earlier than the proximal processes. However, many of the human cases stated in here are long standing deafness which is quite different from the acute exposure (Hurizing and de Groot, 1987). In clinical studies, aminoglycoside induced peripheral nerve symptoms have not been reported. However, peripheral nerve symptoms which include paraesthesia, motor weakness or sensory impairment had been associated with the use of the penicillins, sulfonamides, chlorophenicol, colestin, metronidazole, isoniazid, ethionamide and dapsoma (Snavely and Hodges, 1984).

Streptomycin is the only aminoglycosides in which

its effect on the conduction of the action potential had been study. Sokoll and Diėcke (1969) investigated the effect of streptomycin on the A potential of frog sciatic nerve conduction. A local anesthetic-like action was observed. In their studies, 400 mg/l of streptomycin was found to resemble the effect of 0.002% of lidocaine or 0.003% of procaine. The effect included depression of action potential, conduction velocity and slope of depolarization. The depression of action potentials ranged from 10 to 25% when the concentration of streptomycin increased from 400 to 3200 mg/l and the slope of depolarization was from 87 to 67% of the original value. The decrease in conduction velocity averaged 10% in a concentration of 400 mg/l. Little change was observed when the concentration decreased to 50 mg/l. The effect was due to the changes of the sodium conductance of nerve during excitation. Since the rate of change of the membrane voltage during excitation was a function of the sodium current or sodium conductance, a decrease in sodium conductance would reduce the slope of the action potential and the size of the action potential. The peak membrane potential during excitation will not approach the sodium equilibrium potential as closely as with normal sodium conductance. The decreased conduction velocity was a direct result of the reduced slope of the rising phase of the action potential. These changes were very rapid in onset and

readily reversible if the exposure was of short duration. However, the authors failed to mention the % of the recovery in their studies. Furthermore the number of animals involved had not been reported and no statistical test had been presented.

One of the probable mechanism that neomycin may affect the nervous conduction is by changing the permeability of Na^+, K^+ and Ca^{2+} ions which associate with the action potential in the nerve conduction. The rising phase of the action potential occurs as a result of an increased membrane permeability toward Na^+ which causes the membrane potential to move toward the equilibrium potential for Na^+. Within a few milliseconds after the initiation of the action potential, the permeability of K^+ begins to rise, an event that counteracts the increasing permeability toward Na^+. As a result, the membrane potential begins to return toward resting condition. The permeability to Na^+ again returns to resting value and the falling phase occurs. The refractoriness accompanying the action potential is determined by the inactivation of the sodium and an increase of potassium membrane permeability. Any action or mechanism that would affect the permeability of Na^+ and K^+ would result in change in amplitude as well as the conduction of the nervous impulse.

Molecular models for impulse initiation and propagation had been proposed by many investigators. It

is generally accepted that the ionic channels or pores that underlie membrane permeability are macromolecules. They are most probably single proteins or protein complexes that form aqueous pores within the lipid bilayer of the membrane to allow passage of a given ionic species. The outer portion of the channel which faces the extracellular environment most probably have a selectivity filter of unknown composition that can recognize specific cations or anions. Within the channel itself, some form of sensor factor is present that can recognize the electric field surrounding the ionic pore and can rapidly induce a conformational change in the macromolecule, including the portion that is responsible for the gating of ions.

Na^+/K^+- ATPase is an enzyme believed to be present in all living cell membranes. The primary function of the enzyme is to maintain the transport of cations so that a proper ionic gradient inside and outside of the cells are established. It has been suggested that it is one of the macromolecular constituents of nervous tissue that plays a critical role in the function of the nervous system. The Na^+-K^+ pump is especially active in the axolemma immediately after the action potential. Its presence is essential for the maintenance of both the Na^+ and K^+ concentration gradients across the axolemma. During the action potential, a small amount of Na^+ leaks into and a small amount of K^+ leaks out of the neuronal

cytoplasm. Although relatively insignificant after one or two potentials, an enormous alteration of the intracellular Na^+ and K^+ concentration would ensue if not constantly corrected by the Na^+-K^+ pump.

If neomycin acts directly on the nervous conduction, a local anesthetic-like action should be demonstrated as was reported by Sokoll and Diecke (1969). The blocking effect of the local anesthetic drugs are through the blockade of the sodium current and to a lesser extent the potassium current. The mechanism underlying the blockade of sodium current is quite different from the action of tetrodotoxin. They are active when applied on the internal surface of the membrane, or when applied externally, they can diffuse through the membrane and reach the receptor site within the sodium channel because of their high lipid solubility. The anesthetic molecule gains access to the receptor when both the inactivation and activation gates are open. The local anesthetic receptor interaction produce conformational change of the channel proteins and consequently, the probability of closure of the inactivation gate is enhanced. The binding of the molecule is stronger when the sodium channel is in the inactivated state. As a result, the flow of sodium ions through the channel is prevented (Khodorov, 1981; Mitolo-Chieppa and Carratu 1983).

However, the result obtained here does not

parallel with the study by Sokoll and Diecke (1969), the concentration of neomycin used in here (3500 mg/l) is much higher than their study. If a dose-dependent effect is resulted, the amplitude of the action potential should decrease by more than 25% and the conduction velocities should decreased by more than 10% in the A potential. The occurrence of the depression should be quite rapid in onset and readily reversible. However, the present study does not reveal any significant changes in the amplitudes and the conduction velocities. The conduction velocities recorded were steady all the time. It did not show any change in conduction velocities with time.

The difference in action of neomycin with the local anesthetic may be the water soluble polycationic aminoglycoside can not gain access into the hydrophobic receptor site of the channel. From the molecular point of view, the ionic channels are composed of integral proteins, hydrophobic segment situated deeply in the lipid interior of the membrane and hydrophilic segments protruding out of membrane plane into the water phase. Neomycin must diffuse into the lipid membrane matrix to reach the site of action.

Another probable mechanism by which neomycin may act on the nerve fibre is by disturbing the metabolism of the phosphoinositides in the nervous system. The

inositol-lipid pathway had been proposed as another second messenger system. In the nervous system, hydrolysis of inositol lipids generates two second messengers, IP_3 and diacylglycerol which regulate the membrane potential in tail motor neuron of Aplysia (Sawada, Cleary and Byrne 1989).

There is clear evidence that neomycin interferes with the hydrolysis of the phosphoinositides into inositide trisphosphate. Neomycin occupies calcium binding sites provided by negatively charged phosphate groups of the polyphosphoinositides in the membrane. The neomycin-bound phosphoinositides is a poor substrate for the dephosphorylating enzyme and the dephosphorylation, phosphorylation cycle is disturbed (Schacht, 1976). Next question is whether the metabolism of the phosphoinositides affect the conduction of the nervous impulse.

Polyphosphoinositides are localized predominately in myelinated nerves (Eichberg and Dawson, 1965). Physical and chemical properties of phosphoinositides indicate that these lipids may be capable of controlling ion permeability in membranes. Phosphoinositides e.g. inositol trisphosphate complexes interact readily with divalent metal ions such as calcium and magnesium. In the absence of divalent metal ions, these lipids are largely water soluble but become water insoluble upon the addition of metal ions. Phosphoinositides complexes

interact readily with a variety of proteins forming in most cases water soluble complexes. (Hendrickson and Reinertsen, 1971).

Hawthorne and Kai in 1970 suggested that the hydrolysis of inositide trisphosphate to inositide bisphosphate or monophosphate in the nerve axonal membrane results in a release of bound Ca^{2+} ions which opens up pores with increased permeability to monovalent ions. In Hendrickson and Reinertsen studies, conversion of triphosphate to bisphosphate would result in a release of 70% of bound Ca^{2+} and a 25% decrease in ligand charge. The net increase in positivity of the membrane would favor an increase in K^+ over Na^+ permeability implicating this mechanism in the falling phase of the action potential. On the other hand, changes in membrane structure might lead to increase negativity at the channel sites despite an overall increase in positivity of the membrane. This mechanism would favor an increase in Na^+ over K^+ permeability as in the rising phase of the action potential. Since in the absence of divalent metal ions, phosphoinositides or phosphoinositide proteins complexes are largely water soluble, hydrophilic pores of increased local negativity could be formed under these conditions. For monovalent ions in biological systems, pore charge is of even greater importance in control of selective ion permeability.

High metabolic activity of the inositide had been shown both in nerve cell bodies and highly in poorly myelineated nerve fibres. White and co-workers (1974) demonstrated that electrical stimulation results in increased incorporation of phosphate into inositol trisphosphate in poorly myelineated nerve fibres. In vagus nerve, it gives the most clear cut result as the passage of each impulse in such fibres is associated with much larger ion movement to per unit volume than in myelineated fibres. Tetrodotoxin which selectively blocks the outside of the channels through which sodium ions normally flow during the rising phase of the action potential has no effect on phospholipid labeling in vagus or phrenic nerve. It appears to bind to cholesterol rather than other membrane lipids as measured by the monolayers technique and does not affect active sodium transport at the concentration normally used. Salway and Hughes (1972) suggested that polyphosphoinositides are not involved in the control of the sodium channel or else tetrodotoxin would be expected to produce a decrease of ^{32}P incorporation into these lipids, or the changes of the polyphosphoinositides may be taken place at the inner end of the sodium channel where ATP is available and external tetrodotoxin would have no effect.

Ouabain which can inhibit Na^{+}-K^{+} ATPase in axolemma and plasma membranes of Schwann cells, at concentrations

which block the action potential in vagus nerve, had no effect on the triphosphate or bisphosphate. Increased abeling of phospholipid was found. (White, Schellhase and Hawthorne, 1974). On the basis of work with the iris and other tissues, Akhtar and Abdel-Latif (1982) postulated that the rephosphorylation of 1,2 diacylglycerol into phosphatidic acid and phosphoinositides could be associated with Na^+ outflux via the Na^+-pump mechanism.

In clinical conditions, the lack of inositol and resulting metabolic disturbances may be responsible for the nerve conduction defects in diabetes (Winegrad, 1983). Incubation of isolated sciatic nerve of diabetic rats in a medium containing [^{33}P] orthophosphate result in decrease in labeling of phosphatidylinositol and substantial changes in the labeling pattern of phosphatidylinositol phosphate and bisphosphate from that of controls. The ratio of label in these polyphosphoinositides decreased from 2.5 for normal myelineated nerve to about 1 for diabetic nerve and 2.8 in the poorly myelineated fibres within 45 minutes of incubation (Natarajan et al., 1981).

These studies pose the idea that polyphosphoinositides may be involved in the control of membrane permeability during axonal conduction. Alternation in their relative amount or turnover rates

would result in changes in the amplitudes of the potential as well as the conduction velocities.

The present investigation using neomycin as a model for the alternation in the metabolism of phosphoinositides revealed that there is no significant difference in the amplitude as well as the conduction velocities in the myelineated and unmyelineated nerve fibres. Slowing of the conduction velocity was not shown after exposing the nerve for 60 minutes in a medium of 3500 mg/l of neomycin in Krebs solution. Flushing the nerve with Krebs solution and continuous monitoring for 30 minutes show no apparent changes in the conduction velocity.

There are no significant changes in the A and C potential in tail ventrolateral tail nerve during the application of neomycin solution. In the frog sciatic nerve studies, there is no significant difference in the peak potential before and after the exposure with neomycin. The reason may be 1. the acute effect of the neomycin may not have any apparent effect of the phosphoinositides metabolism; 2. as proposed by Salway and Hughes (1972), the changes of the polyphosphoinositides may be taking place at the inner end of the channel and neomycin cannot enter this space; 3. the metabolism of the phosphoinositides is not involved in the conduction of the nervous impulses.

Another possible mechanism that neomycin may affect

the nervous impulses is through its competing action with calcium ions. It had been well established that neomycin competes with the calcium in the calcium binding sites at the neuromuscular junction (Kubikowski and Szreniawski, 1963; Prado et al 1978; Singh et al 1978). In acute exposure, it also competes with Ca^{2+} for membrane binding sites on the hair cells of the acoustico-lateralis systems that activate the transduction process (Sand, 1975; Yanagisawa et al. 1977; Kroese and Van den Bercken, 1980 and Williams, Zenner and Schacht, 1987). Experiments on single fibres of squid axon, isolated fibres in the frog sciatic nerve and node of Ranvier of frog sciatic nerve (Frankenhaeuser, 1957; Frankenhaeuser and Hodgkin, 1957; Hille, 1968) indicated that the action potential amplitude was smaller when $[Ca]_o$ was low and the threshold increases with the increase of $[Ca]_o$. The relation between the threshold and the log $[Ca]_o$ was nearly linear, with a slope of about 9 mV per fivefold change in $[Ca]_o$.

The effect of calcium ions can be explained by the fact that calcium ions are adsorbed at the outer edge of the membrane and thereby create an electric field inside the membrane which adds to that provided by the resting potential. Adsorbed calcium ions might alter the distribution of other changed particles inside the membrane without changing the overall potential

difference between inside and outside (Frankenhauser and Hodgkin, 1957). Another suggestion is based on the similarity between the action of calcium and changes of membrane potential. The increase in permeability occurs because depolarization removes calcium ions from sites or carriers in the membrane (Frankenhauser and Hodgkin, 1957). It was later suggested that Ca^{2+} acts on the excitability of nerve through the electrical screening of negative charges which are assumed to be fixed on the external surface of the membrane (McLaughlin, Szabo and Eisenman, 1971). This hypothesis would in the absence of further complication, require that cations of the same valence have essentially the same effectiveness. Studies by Brismar (1980) showed that there is greater effectiveness of calcium compared to other divalent cations belonging to the same group of elements. It is assumed that the calcium ions affect the position of Na^{+} permeability curve on the potential axis because of an interaction between calcium and fixed negative charges at the external membrane surface. D'Arrigo (1974) in an investigation of the action of different divalent cations on the threshold for excitation in crayfish axons found that the pH influences the effectiveness of the different ions.

The results in the present study does not parallel with the effect of Ca^{2+} free solution on nervous conduction which could be expected a decrease in the

amplitude of the action potential. It may appear that the effect of neomycin on the nerve fibres behaves differently to that of the depletion of calcium ion on the nerve fibre.

1.5 EFFECT OF HYPERKALEMIA IN THE AMPLITUDES AND CONDUCTION VELOCITIES OF NERVOUS IMPULSE IN THE NERVE FIBRES

Potassium is the principal cation of all cells and its main functions are 1. contribution to cell osmotic pressure. 2. activation of enzymes and 3. the potential across cell membranes.

In the resting state of the nerve cells, the steady Na^+ influx is balanced by a steady K^+ efflux. The resting potential is close to the Nernst potential of K^+. Changes in extracellular potassium concentration results in changes in membrane potassium.

Acute elevation of plasma potassium ions will lead to increased excitability (i.e. reduced threshold) in nerve fibres. It had been shown by Seneviratne and his co-workers (Seneviratne, Peiris and Weeraswiya, 1972) that acute elevation (up to 8 mM) of plasma potassium ions in cats leads to an increase in excitability of myelinated fibres in the tibial nerves. Further increase in the extracellular potassium ion will result in reducing the excitability of the nerve fibres instead of

increasing it. Huxley and Stampfli (1951), working on single isolated frog myelinated fibres, demonstrated that a depolarization conduction block was produced when the extracellular potassium concentration was between 10 and 20 mM. Frog motor neurons are inactivated by an extracellular potassium concentration of 12 mM in an isolated spinal cord preparation (Matsura, Kawaguchi, Ichiki, Sorimochi, Kataoka and Inomya, 1969). In Huxley and Stampfli's studies, it took only 1 second or less for a change in bath composition to alter the resting membrane potential. Hence in isolated single myelineated fibre, the ion fluxes between the nodal periaxonal spaces and the external bath equilibrate very rapidly. Krnjeviv (1954) noted that desheathed frog sciatic nerves were blocked by 120 and 60 mM external potassium concentration in about 25 and 55 seconds respectively.

The blocking time in the present experiment is of the order of several minutes which is quite comparable to the study of Weerasuriya (1987) who showed an average blocking time of 7.9 $\pm$0.3 minutes. The difference between blocking times of single fibre and that of desheathed whole nerve may be due to the disruption of periaxonal diffusion barriers during the isolation of single fibre or the presence of a significant impedence to free diffusion of ions within the endoneurial stroma in whole nerve. Early studies of the compound action potential suggested that the sequence of nerve block was

related partly to size, smaller fibres tended to be blocked before larger fibres (Gasser and Erlanger, 1929). It was thought that the large surface / volume ratio of smaller axons gave the drug quicker access to the seat of action in the protoplasm (Gasser and Erlanger, 1929). It was however later shown that the membrane channels rather than axoplasm are the real seat of conduction block (Hodgkin and Huxley, 1952).and that local anesthetics block the channels by a mechanism that does not implicate axonal diameter.

The slower blocking effect of potassium on the C potential in here may be due a larger larger numbers of unmyelineated nerve fibres in the whole nerve. Langford (1983) showed that the ratio of unmyelineated to myelineated nerve fibre was more than 70% in cat motor, cutaneous and articular nerves. The unmyelineated fibres are situated more centrally. It may thus give the endoneurial extracellular space better stability or it may be due to the different in morphology of the myelineated and unmyelineated nerve fibres. The Na^+ and K^+ channels are predominately located in the node of Ranvier while the ionic channels in the unmyelineated nerve fibres spread widely within the nerve fibres. Bundles of unmyelineated nerve fibres are enclosed by the Schwann cell which gives them better stability. Elevation of extracellular potassium concentration, before leading to a conduction block, produces a

decrement of the amplitude of action potential. The decrease in height of the compound action potential, however cannot be taken as an indicator of the proportion of fibres that have been inactivated.

The arrangement of the present experiment has its own limitation. It is assumed that the action of the neomycin is confined to the immersed portion of the nerve fibre. The exact diffusion can only be shown by radioactive labeling studies or fluorescent studies to study the diffusion rate of substance of similar molecular weight. Since the amplitude of the A and C potential fluctuate during the whole course of monitoring. Unitary single nerve fibre recording may give a better indicator in this aspect. As far as the effect of the neomycin on the metabolism of the phosphoinositides in the nervous tissue is concerned, radioactive labeling may give a more direct measurement of the effect of neomycin on the metabolism of the phosphoinositides.

SECTION VI:- CONCLUSION

CONCLUSION:-

The results from the present study indicate that with the present experimental arrangement, neomycin does not produce any acute functional impairment of nervous conduction in the myelinated and unmyelinated nerve fibres, even in the present of a fairly high concentration (3500 mg/l). This parallel with the finding obtained by Chan and his co-workers (1985) in the rabbit sural nerve with lower concentration (350 mg/l to 1400 mg/l). The mechanism of action of neomycin on nerve fibre behaves quite different to the neuromuscular junction in which the aminoglycosides compete with calcium ions for the same receptor on the nerve terminal membrane and produce complexes that are incapable of transmitter release (Vital-Brazil and Prado-Franceschi. 1969; Yamada et al 1986). It also works differently from the lateral line organ in which neomycin competes with calcium ion for membrane binding sites on the hair cells of the acoustico-lateralis systems that activates the transduction process (Sand, 1975; Yanagisawa et al., 1977; Kroese and Van de Bercken, 1980 and Williams et al, 1987). In chronic exposure, it causes degeneration of the hair cells in the inner ear (Hawkins, 1976) and reduction of responsiveness in touch corpuscles (Baumann et al., 1986).

A local anesthetics like action as reported by

Sokoll and Diecke (1969) cannot be reproduced in here. It is quite likely that neomycin behaves differently from the local anesthetics on the effect of the blockade of sodium and potassium currents.

10 mM K_2SO_4 in Krebs solution shows a complete blockade of the A potential in 5-10 minutes in the present experimental arrangement. However, in the unmyelinated nerve fibre, the blocking effect of the C potential is slower and smaller than the myelineated nerve fibre. It may be because the potassium and sodium channel in the unmyelinated nerve fibre are surrounded by a layer of Schwann cell which act as an diffusion barrier.

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