ROLE OF SOME HUMORAL FACTORS ON NERVE MUSCLE PREPARATION AND ITS RELATED ELECTROPHYSIOLOGICAL PHENOMENA

A THESIS
SUBMITTED FOR PARTIAL FULFILMENT OF THE MASTER DEGREE IN PHYSIOLOGY

BY

KAMAL MAHDI HASSAN
M.B.B.CH

Under Supervision of
Prof. Dr. MAHMOUD ELKAREMI
Professor and Chairman of Physiology Department
Faculty of Medicine
Ain Shams University

Dr. MOHAMMED Ismail Elgouhary
Assistant Professor of Biophysics
Al Azhar FAculty of Science.

Dr. MOHAMMED NOUR ELDEEN HAFEZ
Lecturer of Physiology

Faculty of Medicine
Ain-Shams University
1 9 8 4

ACKNOWLEDGEMENT

I wish to thank Professor Dr. Mahmoud El-Karemi Professor and Head of Physiology Department who suggested and planned this work. His keen supervision, continious guidance and generous encouragement.

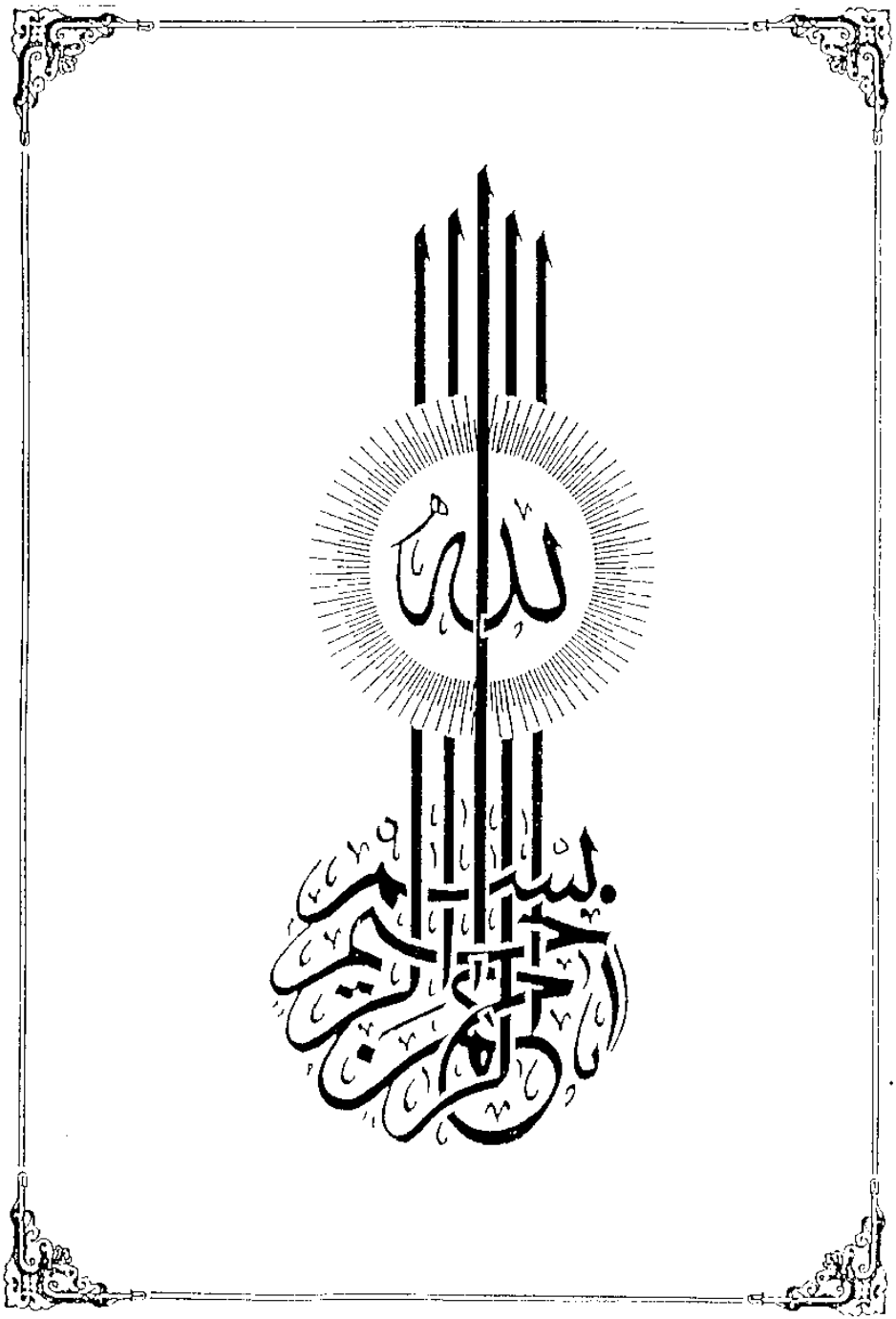
My optimal gratitude is due to Dr. Mohammed Ismail El Gouhary Assistant Professor of Biophysics Al Azhar Faculty of Science.

I wish to express my great thank and sincere appreciation to Dr. Mohammed Nour Eldeen Hafez for his continious supervision, guidance and without him this work was impossible.

I am grateful to Dr. Salah Abou Shanab for his kind help and support.

My sincere and great thanks are due to the German Academic Exchange Service for their sponsering my scolarship.

Finally I would like to thank all the staff of the Physiology Department Ain Shams Faculty of Medicine for their kind help.



CONTENTS

INTRODUCTION

The relationship between the nerve, neuromuscular transmission and muscle contraction were investigated before. The present work was planned to show the effect of some humoral factors on conduction velocity of the sciatic nerve,neuromuscular transmission of the gastrocnemius sciatic nerve prepartation and muscle contraction of the rectus abdominis muscle of the frog. The effect of these humoral factors may be benifical for medical purposes.

Among humoral factors chosen, are kallikrein and kinin as an example of kallikrein-kinin system which plays a role in many physiological processes in our bodies e.g.(vasodilatation accompanies salivary and pancreatic secretion). Trayslol as an example of kallikrein inhibitor. Insulin which is the anabolic and storage hormone. Dexamethazone which is one of the adrenaocortical hormones which have many important physiological as well as pharmacological actions inside the human body e.g. hyperglycaemic effect, protein catabolic effect and a diuretic effect. Frog tissue was used because it can function isolated without blood supply and can get oxygen by diffusion from the surrounding medium.

INTRODUCTION

REVIEW OF LITERATURE

THE NERVE

The function of the nerve is to convey messages within as well as to and from the C.N.S.

A nerve consists of very many nerve fibres, which convey messages to different places. Nerve fibres are well insulated from one another by cellular neurlemma, or sheath of schwan cells.Between neurolemma and the nerve fibre sometimes a myelin sheath, consists of lipids and proteins, thus, nerve fibres referred to as myelinated or unmyelinated.

Hodking (1964) stated that nerves can be stimulated by different mechanisms but the best stimulus is the electrical one.

Conduction Velocity

Hodking (1964) pointed that if a long nerve is stimulated around mid length, the impulse starts at the cathode but propagates toward both ends of the nerve.

Propagation means that the impulse doesn't remain stationary at the point of origin it travels along the nerve fibre in both dierctios.

Hodking (1964) stated that the speed of conduction depends on the following factors:

1) *Fibre diameter. Large fibres conduct impulses faster than small fibres.*

2) *The temperature of the nerve.Fibres conduct at higher speed if the temperature is high, and lower speed if the temperature is low. Indeed extreme cooling can entirely block the conduction or propagation of the nerve impulse. This is applied clinically by use of cold to anaesthetize a region of a person's skin. This is due to partial block of the nerve fibres responsible for conduction of pain evoked impulses.*

3) *The presence or absence of myelin:*

Conduction in the unmyelinated fibres is slower than in myelinated fibres.

Hodking (1964) concluded that conduction velocity is one criterion by which we can identify different fibres in a nerve. He also pointed that the conduction velocity of the myelinated fibres of the mammals is related to the diameter by a factor of 5 for longer fibres and of 4 for the smaller ones. It means that if we devided the conduction velocity in meters/second by either 4 or 5 we have a fairly accurate idea of the external diameter in (microns) of a given nerve fibre.

Hodking (1964) stated that fibres in mammals conduct at a much faster speed than those in cold blooded animals (e.g,the frog). In the frog, fibres with a diameter of 11-18.5 microns conduct at 17-42 meters/second. The slower conduction velocity in cold-blooded animals may be due to the lower temperature of these animals.

Neuromuscular Transmission

A nerve impulse affects the post-junctional muscle region and this causes a propagated muscle impulse. It was widely assumed that the transmission process was caused in some way by the action currents of the nerve impulse.

Dale, Feldberg and Vogt(1936) observed that when the motor nerve to a perfused muscle was stimulated, there appeared a substance in the perfusate which had the physological characterastic of acetylcholine.

Brown , Dale , Feldberg (1936) and Brown (1937) observed that when acetylcholine was injected into the arterial supply close to the muscle, a contraticle resonse consisting of brief tetanus developed. In a complex event like transmission, a substance which appears in a perfusate may play a role in many phases of excitation or recovery.Studies on the end-plate potential clarified many of these points. These potentials are set up by every nerve impulse on reaching the junction and are recorded as focal depolarization of the post-junctional muscle membrane. When this depolarization reaches a critical level, it sets up propageted impulses in the muscle membrane surrounding the junction. The end-plate potential in normal muscle is largely obscured by these impulses but can be made evident when neuromuscular block is produced by curarization or fatigue.

The nerve fibre branches at its end to form a complex of branching nerve terminals called the end plate, which invaginates into the muscle fibre but lies outside the muscle fibre plasma membrane.The entire structure is covered by one or more schwan cells that insulate the end-plate from the surrounding fluids. **(Guyton,1981).**

The invagination of the membrane is called the synaptic gutter or synaptic trough, and the space between the terminal and the fibre membrane is called the synaptic cleft. The synaptic cleft is 20 to 80 nanometers wide and is filled with a gelatinous ground substance through which diffuses extracellular fluid. **(Guyton,1981).**

At the bottom of the gutter are numerous folds of muscle membrane which form the subneural clefts that greatly increase the surface area at which the synaptic transmitter can act. In the axonal terminals, there are many mitochondria that supply energy mainly the excitatery transmitter acetylcholine that in turn excites the muscle fibre. The acetylcholine is synthesized in the cytoplasm of the nerve terminals which is rapidly absorbed into many synaptic vesciles, approximately 300,000 of which are normally in all the terminal of a single end-plate. In the matrix of the subneural clefts are large quantities of the enzyme cholinesterase which is capable of destroying acetylcholine. **(Guyton,1981).**

The sequence of events in the neuromuscular junction is as follows:-

nerve impulse----transmitter----end-plate potential----muscle impulse. The latter then initiates the activation processes as it passes along the muscle fibre. The end-plate potential depolarizes the region around the junction and thus sets up the muscle impulse when a critical level is reached as stated by Eccles, Katz, Kuffler(1941) and Kuffler (1942). If the transmitter action has been reduced, for instance by fatigue or by curarization, then the critical depolarization for muscle fibre excitation will not be reached and end-plate potential alone, without a muscle impulse (and twitch) will be detected at the junction. Before such paralysis is reached, the end-plate potential may be reduced to about 1/3 of its orginal height because of this safety factor, the effects of subparalytic doses of drugs like curare can be detected electrically in single units well before block.

Kuffler (1945) reported that while the end-plate region of the muscle fibre shows a striking specific excitabity to applied drugs like acetylcholine or nicotine, there is no detectable difference in the electrical excitabilty between the end-plate and other regions of the muscle.

Cowan (1940) Brown , Dale and Feldberg (1948) reported the effects of cholinesterase inhibitors,particulary eserine

provided some of the earliest evidence for the existence of a cholinergic mediator at neuromuscular junction.

By the use of mechanical recording methods,it has been shown that the principal effects of anticholinesterases are to:-

1) Cause an increase in twitch response to a single nerve stimulus, the increased response being due to repetitive firing of the muscle;

2) increase the depression of response to high frequency stimulation of the nerve; and 3) alter the response to injected acetylcholine. In contrast to the above the response to dierct stimulation is not altered.

Acetylcholine is capable of exciting the end-plate region of the muscle, as are a number of other choline esters and nicotine. Acetylcholine is effective in very small concentrations, and application of 1×10^{-6} to frog muscle or intraarterial injection of as little 1 microgram to cat muscle causes a train of muscle discharges as reported by Brown,Dale and Feldberg (1940).

Cowan (1936) detected that the depolarizing effect of acetylcholine is confined to the end plates.

Artificial stimulation of the nerve fibres greater than 100 times per second for several minutes often dimishes the number of vesicles of acetylcholine released with each impulse so that

the impulses often fail to pass into the muscle fibre. This is called fatigue of the neuromuscular junction. del Castillo and Stark (1952) reported that Ca^{+2} facilitates release of acetylcholine from the presynaptic terminals. Del Castillo and Engbaek (1954)reported that Mg^{+2} ions act in opposite dierction by reducing acetylcholine output.

Drugs that block transmission at the neuromuscular junction:-

Brizin and Zupanic (1956) reported that curare prevents the passage of nerve impulse from the end-plate into muscle. They suggested that curare competes with the cationic head of acetylcholine for the anionic centres of the receptor, so that acetylcholine can not increase the permeablity of the membrane sufficientely to initiate the depolarization wave.

In the Cat,Harvey (1939) produced neuromuscular block with procaine without appreciably changing the contraction of the muscle to dierct stimulation.

Guyton and McDoland (1941) found that bolutinus produced neuromuscular block.

Neuromuscular blocking agents are used to produce muscle relaxation during surgical operations and reduce muscle movements during electroconvulsive treatment of pyschotic patients.

Disorders of neuromuscular junction:

Myasthenia gravis:(Guyton,1981).

Myasthenia gravis results from a defect in neuromuscular junction. The neuromuslar junction is unable to transmit signals from the nerve fibres to the muscle fibres. Here the number of the subneural clefts in the synaptic gutter is reduced and the synaptic cleft is widened as 50%. Also the antibodies that attack the muscle fibre have been demonstrated in the blood of many of those patients. Therefore it has postulated that it is an autoimmune disease in which the patients developed antibodies aganist their own muscles and the antibodies in turn have damaged the muscle fibres. One of the effects being partial destruction of the receptor membrane of the neuromusculer junction. It was found that the end-plate potential in those patients is reduced, perhaps or probably because of the damaged receptor of the membrane of the junction.

Regardless of the cause, the end-plate potentials developed in the muscle fibre are too weak to stimulate the muscle fibre.

Glucocorticoids aggrevate myasthenia gravis. ***(Laurence,1980).***