INTRODUCTION

Neuromuscular blockings agents are used as valuable adjuvant to general anesthetics, administrated to anesthetized individuals to provide relaxation of skeletal muscles to facilitate intubation, positive pressure ventilation and/or surgery; muscle relaxation does not ensure unconsciousness, amnesia or analgesia.

The characteristics of an ideal neuromuscular blocking agent (NMBA) include aspects such as the short onset of action, the short duration of action, the lack of histamine release and the provision of hemodynamic stability.

The 1st NMBA used in clinical practice was a crude extract of curare by **Griffith and Johnson** in 1942, they suggested that d-Tubocararine (d-TC) was a safe drug to use during surgery, to help providing good muscle relaxation. Yet it showed different disadvantages as cardiovascular instability and marked histamine release. In 1954 **Beecher and Todd** reported a six fold increase in mortality in patients receiving a d-TC versus those who had not received a relaxant. This was related to a general lack of understanding of the pharmacology of NMBA and the importance of antagonizing the residual blockade to avoid its effect postoperatively, as well as due to that the

need for careful monitoring of muscle strength had not been established by that time.

The improvements done in this field made the relaxant an important component of many anesthetics and have facilitated the growth of surgery into new areas with the use of innovative techniques ***(Foldes et al., 1952)***.

So Succinylcholine, depolarizing NMBA introd-uced by **Thesleffs** in 1951 and by **Foldes and colleagues** in 1952 revolutionized the anesthetic practice drastically by providing rapid intense blockade of ultra short duration, thereby greatly easing the maneuver of tracheal intubation under excellent intubating conditions, also usually it has the most rapid recovery ***(Blobner et al., 2000)***. However it has many side effects and should be avoided in some conditions ***(Collons et al., 2000)***.

Numerous synthetic and semi-synthetic non-depolarizing drugs were introduced over the next decades. Development of the intermediate acting NMBA resulted in the introduction of Vecuroniun, an aminosteriod ***(Savage et al., 1980)*** and Atracurium, a benzylquinolinium ***(Stenlake et al., 1983)*** into practice in the early 1980s providing relaxation with a little dependence on the kidneys for elimination, faster onset, a more rapid recovery and a faster and more complete antagonism compared to

the synthetic aminosteriod Pancuronium administrated by and first reported in 1967 by **Baird and Reid**.

The lack of cardiovascular effects of Vecuronium established a bench mark for safety to which new relaxants are still held ***(Savage et al., 1980)***. Degradation of Atracurium by Hoffman elimination removed any important influence of biologic disorders such as advanced age or organ failure on the pattern of neuromuscular blockade ***(Stenlake et al., 1983)***. Also it is characterized by being cardiovascular stable and having a wide safety margin of histamine release ***(Hughes and Chapel, 1981)***.

Rocuronium bromide an intermediate acting non-depolarizing NMBA introduced in 1990 with its rapid onset of effect; So in terms of fascilitating rapid endotracheal intubation, Rocurunium is the 1st non-depolarizer considered to be a replacement of Succinycholine ***(Wierda et al., 1990)***. Also it has a more ideal vagolytic profile ***(Smith and Saad, 1998)*** and with no or minimal histamine release ***(Cook, 2000)***.

In this thesis a comparison between four current muscle relaxants will be held as regards their differences related to the intubating conditions, haemodynamic stability and histamine release.

Aim Of The Work

Neuromuscular blocking agent are important adjuvant to general anesthesia to provide adequate surgical condition and ensure intubation and ease the positive ventilation. Various muscle relaxant have been studied aiming at reaching an ideal one.

The aim of this thesis is to run a comparative study between rocuronium and current muscle relaxants as regards the intubating conditions hemodynamic changes and histamine release.

ANATOMY AND PHYSIOLOGY OF THE NEUROMUSCULAR JUNCTION

Neuromuscular junction (NMJ)

As the axon supplying skeletal muscle fiber approaches its termination, it loses its myelin sheath and divides into a number of terminal buttons or end feet (adult neuromuscular junction) (Fig. 1).

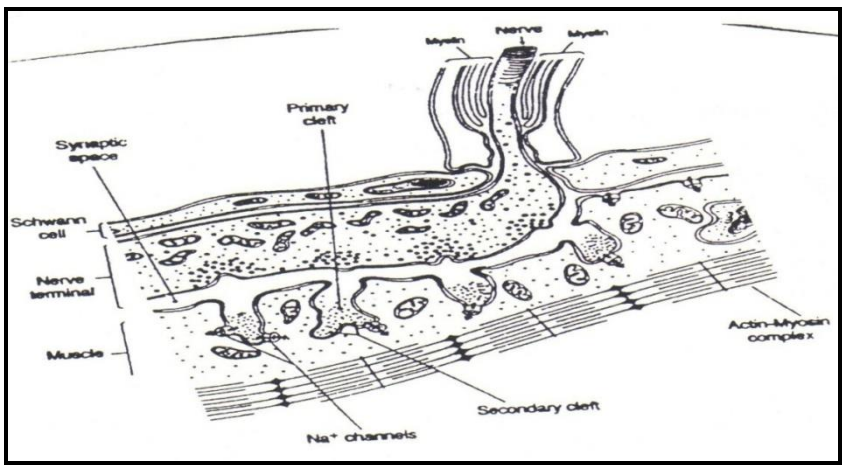

Fig. (1): Adult neuromuscular junction *(Martyn et al., 2000)*.

The end feet contain many small clear vesicles that contain acetylcholine, which is the transmitter at this junction. The ending fits into depressions in the motor end plates which is the nerve ending, the muscle membrane of the end plate is thrown into the junction folds and clefts. The space between the nerve and the thickened muscle

membrane is comparable to a synaptic cleft as synapses. The whole structure is known as neuromuscular or myoneural junction ***(Ganong, 1997)***.

Motor units

Striated muscles are innervated by the lower motor neurons, which pass uninterrupted from the ventral horn of the spinal cord to the muscles. Each motor neuron innervates many muscle fibers and the neuron together with its muscular fibers form a functional group known as a motor unit .The number of muscle fibers in a unit depends on the function of the muscle in question, varying from less than 10 in extra ocular muscles to more than 1700 in postural muscles, the more delicate the movements, the fewer muscle fibers per motor neuron ***(Buchtal, 1960)***.

On the other hand a motor nerve enters the skeletal muscle and branches a variable number of times depending on the function of the muscle. Most human muscle has only one neuromuscular junction per muscle cell usually located near its mid portion, this type of muscle cell is called twitch fiber (with only one endplate). But there is an exception of about 2%of the muscle which include some extra ocular fascial and intrinsic laryngeal

muscles which have multiple innervations, this type is called tonic muscle fibers (with more than one endplate) ***(Franzini and Jorgensen, 1994)***.

The twitch type of muscles which is the majority, can be divided into slow twitch red fibers (e.g., the adductor pollicis) and fast twitch white fibers (e.g., tibialis anterior, diaphragm). Slow twitch fibers tend to have a large aerobic capacity, slow contraction and relaxation times, and resistance to fatigue. On the other hand, fast twitch fibers exhibit limited aerobic metabolism, have fast contraction and relaxation times, and are not resistant to fatigue ***(Bowman, 1990)***.

Stimulation of the nerve causes all muscle cells in the motor unit to contract synchronously, which is called fasciculation ***(Durant and Katz, 1982)***.

The terminal portion of the axon (the synapse) contains vesicles designed for the production, storage and release of acetylcholine. The synapse is separated from the endplate of the muscle fiber by a narrow gap (the synaptic or junctional cleft) ***(Bevan et al., 1988)***. It is filled with collagen structure named the basement membrane. To this membrane deep in the clefts is attached most of acetylcholinesterase present in the NMJ. The primary and secondary clefts between the folds in the

muscle membrane have large surface area. The folds and their shoulders are densely populated with acetylcholine receptors (Fig. 2).

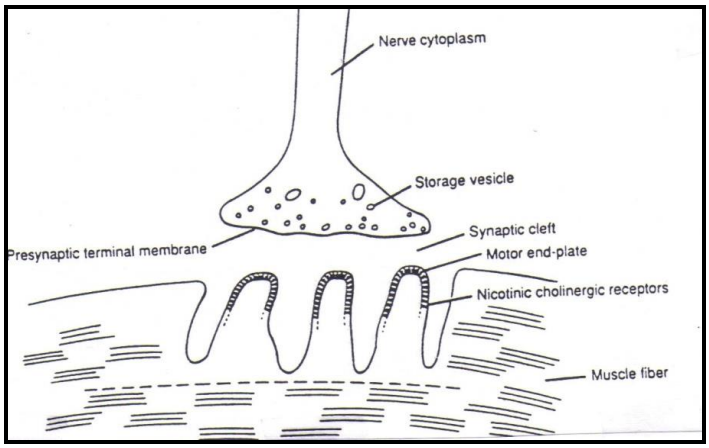

Fig. (2): The neuromuscular junction *(Drachman, 1978)*.

Each endplate has 10^6-10^7 nicotinic receptors ***(Peper et al., 1982)*** or about 5 million receptors per junction as mentioned by ***Martyn et al. (2000)***.

The peri-junctional zone contains a smaller density of acetylcholine receptors and instead a high density of sodium channels. The admixture transforms depolarization into a wave that travels along the muscle to initiate muscle contraction ***(Betz et al., 1984)***.

PHYSIOLOGY OF THE NEUROMUSCULAR JUNCTION

The axon of the motor nerve carries not only electrical signals from the spinal cord to the muscles but also all the biochemical tools needed to transform the electrical signal into chemical one***(McArdle,1984).***

Sequence of events of neuromuscular transmission

The events occurring during transmission of impulse from the motor nerve to the muscle are similar to those occurring in other synapses. When the impulse arrives at the motor terminals, it increases the permeability of nerve ending to calcium ions which enter the ending and trigger a marked increase in the exocytosis of acetylcholine-containing vesicles. The released acetylcholine diffuses through the junctional cleft, and then combines with the post-junctional cholinergic receptors on the folds of the motor end plate (MEP). The complex formed by acetylcholine and the membrane receptors increases the permeability of MEP to sodium and potassium. The resultant influx of sodium produces a depolarizing potential called the end plate potential ***(Standaert, 1994)***.

This current depolarizes the adjacent muscle membrane to its firing level. Action potentials are generated on either side of the endplate and are conducted away from it, in both directions, producing a muscle action potential ***(Zimmermann, 1988)***. The sodium influx initiates Ca^{2+} release from the sarcoplasmic reticulum which in turn activates actin-myocin mechanism within myofibrils, leading to contraction ***(David et al., 1988; Catterall, 1995)***.

The acetylcholine immediately detaches from the receptor and is destroyed by acetylcholinesterase enzyme present in the cleft (Fig. 3).

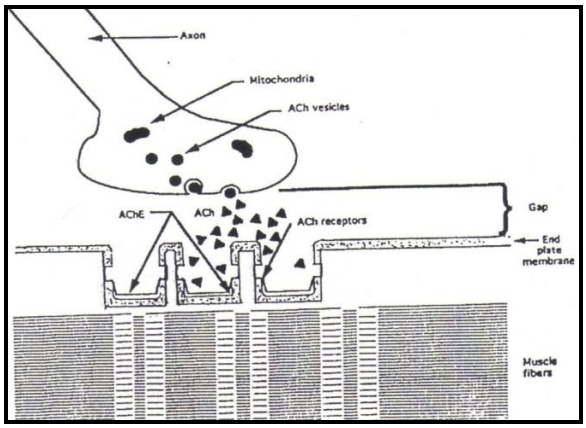

Fig. (3): Diagram shows acetylcholine (Ach) release, diffusion across the synaptic cleft binding to receptors on end-plate membrane and hydrolysis by Acetylcholinesterase (AchE) *(Moss and Craigo, 1994)*.

In brief the depolarizing relaxants cause depolarization of the end plate. Non-depolarizing relaxants

prevent acetylcholine from binding to the receptor. Reversal agents inhibit acetycholinesterase enzyme. The increased acetylcholine can compete with Non-depolarizing relaxants, antagonizing their effects ***(Martyn et al., 2000)***.

Quantal release of transmitter: Acetylcholine (Ach)

Small quanta (pockets) of acetylcholine are released randomly from the nerve cell membrane at rest, each producing a minute depolarizing spike called miniature end plate potential (MEPP) which is about 0.5mV in amplitude. Each spontaneous depo-larizing potential or MEPP is regarded as the effect produced by the quanta of acetylcholine contained within one synaptic vesicle (about 5000 molecules) and this spontaneous vesicle release is far too small to trigger muscle contraction ***(Bowman, 1998)***. When a nerve impulse reaches the ending, the number of quanta released increases by several order of magnitudes, and the result is a large end plate potential that exceeds the firing level of muscle fiber ***(Ganong, 1997)***.

The amount of acetylcholine released by each nerve impulse is large, at least 200 quanta, and the number of acetylcholine receptors activated by a nerve impulse also is large, about 500,000. The flow of ions (mostly Na^+ and Ca^{2+}) through the channels of the receptors causes a

maximum depolarization of the end plate, greater than the threshold for the stimulation of the muscle. The size of quanta of acetylcholine released varies directly with the Ca^{2+} concentration and inversely with Mg^{2+} concentration at the endplate.

So a nerve action potential is the normal activator of neuromuscular transmission but not the trigger. That function belongs to a calcium flux initiated by the action potential. Moreover, the number of acetylcholine released by a stimulated nerve is greatly influenced by the concentration of ionized calcium in the extra cellular fluid and also the length of time during which the calcium flows into the nerve ending ***(Katz and Miledi, 1979)***.

The calcium current begins by the time action potential approaches its maximum and persists until the membrane potential is back to normal by outward fluxes of potassium. Therefore, calcium flow can be prolonged by drugs, which slow or prevent potassium flux ***(Paskov et al., 1986)***.

There are several types of calcium channels of which two seem to be the most important to transmitter release, the fast P-channels and the slow L-channels. The P channels are probably the type responsible for the normal release of transmitter ***(Uchitel et al., 1992)***, found only in nerve

terminals and motor nerve endings and located immediately opposite the crests of post-synaptic membrane. They are voltage-dependent and respond quickly to depola-rization of the nerve ending by an action potential.

Tiny concentrations of bivalent inorganic cations as magnesium, cadmium and manganese block calcium entry through these channels and impair neuromuscular transmission. The P channels are not affected by organic calcium entry blocking drugs such as verapamil, diltiazem and nifedipine which in contrast have profound effects on the cardiovascular system where the L channels are present and so are affected by these Ca^{2+} entry blocking drugs. So these drugs have no a significant effect on the normal release of acetylcholine nor on the strength of neuromuscular transmission ***(Braunwald, 1982)***.

Calcium results in the release of the transmitter through binding with an intermediate protein “calmodulin” which allows the vesicular membrane to fuse with axonal membrane and discharge its content of acetylcholine to junctional cleft ***(Linas et al., 1992)***.

Repeated stimulation requires the nerve ending to replenish its stores of releasable transmitter, a process termed mobilization.If this process fails to occur in an

appropriate rate, comparable to the rate of nerve stimulation, successive reduction in response to repea-ted stimulation will occur ***(Benfanati et al., 1992)***.

Acetylcholine as a transmitter is the key to neuromuscular transmission. In skeletal muscles, the electrical forces produced by the nerve action potential are too weak to initiate a muscle action potential, acetylcholine acts as chemical amplifier to ensure transmission ***(Bowman, 1980a)***.

Acetylcholine has the relatively simple structure, which is the acetylester of choline as shown in Fig. (4).

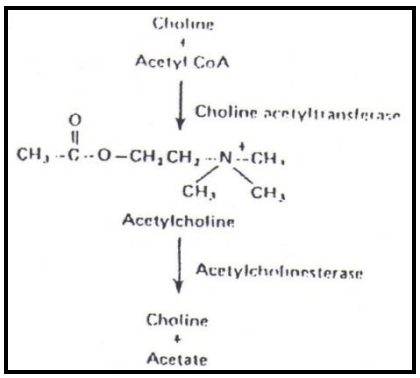

Fig. (4): Biosynthesis and catabolism of acetylcholine *(Ganong, 1997)*.

Acetylcholine synthesis and storage:

Acetylcholine is formed by acetylation of choline within the nerve cytoplasm. It exists in the motor nerve

endings in three forms; storage, reserve, and immediately available forms ***(Elmquist and Quastel, 1965)***.

The storage form of acetylcholine is present in the free cytoplasmic molecules awaiting their packaging into vesicles; it represents about 20% of acetylcholine in the nerve endings. While the reserve acetylcholine comprises the immature vesicles that do not take part directly in neuromuscular transmission and has to be transferred to the immediately available stores prior to release. They are used to cope with repeated nerve stimulation. Finally, the immediately available acetylcholine stores are mature vesicles ready to release their contents. They are most numerous opposite the peaks of the junctional folds and contain about 80% of acetylcholine present in the nerve endings of cholinergic neurons ***(Ganong, 1997)***.

There seem to be two pools of vesicles that release acetylcholine: a readily releasable store and reserve store, sometimes called VP2 and VP1 respectively ***(Linas et al., 1992)***. The vesicles in the former are bound to the active zones and release transmitter. When Ca^{2+} enters the nerve via the P channels lined up on the sides of the active zone, Ca^{2+} activates a protein, synaptophysin in the vesicle wall ***(Valtorta et al., 1988)***, which reacts with the nerve membrane to form a pore, through which the vesicle