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Research article

COMPARISION OF POTENCY OF DIFFERENT TYPES NEURO-MUSCULAR JUNCTION (NMJ) BLOCKERS ON ISOLATED RECTUS ABDOMINIS MUSCLE OF *RANA TIGRINA.*

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ABSTRACT: The most important use of neuromuscular blockers is as adjuvant to general anaesthesia where adequate muscle relaxation can be achieved at lighter plane. They also reduce reflex muscle contraction in the region undergoing surgery and assist maintenance of controlled ventilation during anaesthesia. They are particularly helpful in abdominal and thoracic surgery, intubations and endoscopies, orthopedic manipulation, etc. Thus, the risk of respiratory and cardiovascular depression is minimized, and post anesthetic recovery is shortened. The potency ratio of two commonly used neuromuscular agents depolarizing succinylcholine and non depolarizing pancuronium. Double pith a frog and fasten it to a frog board with ventral side up. The sternum was cut through just above the xiphisternum at its base and a pair of muscle attached to it were dissected out and transferred to a dish containing frog Ringer solution at room temperature. All the drug containing solutions were freshly prepared before the experiments Succinyl choline, Pancuronium (1,10,100µg/ml and 1mg/dl) respectively Acetyl choline (10,100µg/ml and 1mg/dl). Acetylcholine solution in various strength were prepared starting from 0.1% to 0.0001%.NMJ blocker Pancuronium was added to the biophase in addition to selected dose (128µg or 256µg) and the contraction of muscle till the 70-80% of inhibition is produced and the difference from sub maximal contractions. The median ED_{50} was interpolated from the figure taking 50%of inhibition from Height of contraction in mm. The 't' test was performed to compare the ED_{50} value were interpolated from the regression line to find out the ED_{50} of the drug. The median doses (ED_{50}) of both of them were calculated graphically and compared. The mean ED_{50} value of succinylcholine was found to be 1.59 ± 0.08µg (95% confidential limit was from 1.53 to 1.66µg). The ED_{50} of pancuronium was found to be 0.52 ± 0.10µg with 95% confidence limit being from 0.44 to 0.60µg. The ED_{50} value of the two drugs was very significantly different ($P < 0.001$). The potency ratio of pancuronium to succinylcholine was 0.32.

Keywords: Neuro muscular Junction, Rectus abdominais, *Rana Tigrina,* Pancuronium

INTRODUCTION

Neuromuscular blocking agents act peripherally at neuromuscular junction to reduce muscle tone and cause paralysis of skeletal muscles. They are used mainly as a part of balanced anesthesia to provide muscle relaxation during surgery. [(Cammu G. 2001)

The most important use of neuromuscular blockers is as adjuvant to general anaesthesia where adequate muscle relaxation can be achieved at lighter plane. (Fiacchino F 1990) They also reduce reflex muscle contraction in the region undergoing surgery and assist maintenance of controlled ventilation during anaesthesia. (I Wessler and C J Kirkpatrick 2008) They are particularly helpful in abdominal and thoracic surgery, intubations and endoscopies, orthopedic manipulation, etc. Thus, the risk of respiratory and cardiovascular depression is minimized, and post anesthetic recovery is shortened. (N. Döndas, Y. Karatas, A. Dikmen 2004) Neuromuscular junction blocking agents cannot be used to substitute for inadequate depth of anesthesia.

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Among the two types of neuromuscular blockers, the competitive blocker (curare like drugs) acts on nicotinic (N_M) cholinergic receptors at the motor end plate. It reduces the frequency of Na^+ channel opening associated with the receptor, but not its duration or conductance. (Bethesda, 1997) When the magnitude of EPP falls below a critical level, it is unable to trigger the propagated muscle action potential (MPP) and muscles fail to contract in response to nerve impulse. The antagonism is surmountable by increasing the concentration of acetylcholine in vitro and anticholinesterases in vivo. In high doses, they block the channels to produce non-competitive neuromuscular block that is partly reversed by anticholinesterase neostigmine. (Ostergaard D 1989)

The depolarizing blockers, the second type of neuromuscular blockers have also affinity towards the N_M cholinoceptors at motor end plate of neuro-muscular joint. They depolarize the muscle end plate by opening Na^+ channel just like acetylcholine and initially produce twitching and fasciculations. In focally innervated mammalian muscle, stimulation is transient; longer lasting depolarization of muscle end plate produces repetitive excitation of the fiber. (R T. Brittain 1959). In the multiple innervated contracture muscle like rectus abdominis muscle of frog, stimulation is prolonged resulting in sustained contraction. The depolarizing drugs do not dissociate rapidly from the receptor and induce prolonged partial depolarization of the region around the muscle end plate and inactivate Na^+ channels. (Thandla Raghavendra. 2002) Acetylcholine released from motor nerve endings is unable to generate MAP leading to flaccid paralysis in mammals and spastic paralysis in multiple innervated muscles like rectus abdominis in frog and muscles of bird.

As these two types of neuromuscular blocking agents produce entirely two different types of muscle paralysis on rectus abdominis muscle of frog, non-depolarizing neuromuscular blocking agents producing flaccid paralysis and depolarizing agents producing spastic paralysis, the preparation is used in this study to find out the potency ratio of two commonly used neuromuscular agents depolarizing succinylcholine and non depolarizing pancuronium. (Ruben Vardanyan, Victor Hruby 2006)

METHOD (I Wessler and C J Kirkpatrick, 2008, Yuji Nagawa et al, 1971)

The study was approved by the IAEC authorities and it followed the CPCSEA rules on animal protection. Frogs, weighing 150 – 250g, were pithed. Dissection Procedure: Double pith a frog and fasten it to a frog board with ventral side up. The skin of the abdomen was picked up with forceps and cut from above the sternum down to the fork. It was then cut laterally to expose the whole abdomen the recti was seen running on each side of the midline from the base of the pectoral girdle down to the pelvic girdle. The sternum was cut through just above the xiphisternum at its base and a pair of muscle attached to it were dissected out and transferred to a dish containing frog Ringer solution at room temperature. The muscles were divided unless they are very small. Threads were attached to the top and bottom of each piece by purse string suture. The fork end is tied to oxygen tube (pin in the organ bath) with help of a fine thread. Xiphisternum is tied to the free limb of the writing lever. Which is fixed to stand and the tension adjusted such that it gives maximum contractions. Proper tension and magnification was adjusted by altering the height of levers. The capacity of organ bath was 15ml: fluid is taken till it just immersed the tissue. An initial load of 10g was added to stretch the muscle for 45min. The load was attached to the lever at the same distance from the fulcurum is the preparation on other side. This load was raised and lowered as was necessary. All the drug containing solutions were freshly prepared before the experiments Succinyl choline, Pancuronium (1,10,100µg/ml and 1mg/dl) respectively Acetyl choline (10,100µg/ml and 1mg/dl). Acetylcholine solution in various strength were prepared starting from 0.1% to 0.0001%. Dose was added by starting from the highest dilution (10^{-6} solution) and increased in geometric progression (log dose). The dose was increased till there was no further increase or a decrease in the effect with the highest dose. Among these the dose producing sub maximal contractions was selected. The Non depolarizing NMJ blocker Pancuronium was added to the biophase in addition to selected dose (128µg or 256µg) and the contraction of muscle till the 70-80% of inhibition is produced and the difference from sub maximal contractions (inhibition in height of contraction) was recorded.

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The procedure was repeated by NMJ blocker Pancuronium in the dose of 0.1µg, 0.2µg, 0.4µg and 0.8µg respectively. The effect depolarizing NMJ blocker Succinyl choline was added to the biophase in addition to selected sub maximal dose and the contraction of muscle till the 70-80% of inhibition is produced and the difference from selected dose contractions (inhibition in height of contraction) was recorded. The procedure was repeated by adding depolarizing NMJ blocker Succinyl choline in the dose of 0.1 µg, 0.2 µg, 0.4 µg, 0.8µg, 1.6 µg, 3.2µg, 6.4 µg 12.8 µg, 25.6 µg, 51.2 µg, 102.4µg and 204.8 µg respectively. Individual findings are recorded on smoked drum cylinder, fixed with resin (colophony) and the recordings are measured.

RESULTS

The ED_{50} of Succinylcholine was plotted taking log dose of Succinylcholine in the X axis and the Height of contraction in mm in the Y axis. The ED_{50} of Pancuronium, was plotted taking log dose of Pancuronium in the X axis and the Height of contraction in mm in the Y axis. The median ED_{50} was interpolated from the figure taking 50%of inhibition from Height of contraction in mm. The 't' test was performed to compare the ED_{50} value. were interpolated from the regression line to find out the ED_{50} of the drug.

The mean, standard deviation and standard error of the six observations were made and the 95% confidential limit was calculated of the ED_{50} value of pancuronium and succinylcholine. The mean ED_{50} value of succinylcholine was found to be 1.59 ± 0.08 µg (95% confidential limit was from 1.53 to 1.66 µg) while the ED_{50} of pancuronium was found to be 0.52 ± 0.10 µg with 95% confidence limit being from 0.44 to 0.60 µg. The't' test was performed to compare the ED_{50} value, which was found to be highly significant ($t = 20.79^{***}$ and $P < 0.001$)

DISCUSSION

In the present study, potency of two commonly used neuromuscular junction blocking drug of different mechanism of action was compared and the relative potency ratio was calculated. The long acting non-depolarizing (competitive) blocker pancuronium and the depolarizing blocker succinylcholine (suxamethonium) were two drugs taken for comparison in this study. The responses corresponding to the dose were measured from the tracing and were recorded. (W C Bowman.Neuromuscular block. 2006). The upper and lower 30% of the responses were discarded as they did not come in the liner portion of the dose-response curve. The logarithmic transformations of rest of the doses were made. The log dose versus response graph were prepared and regression line (or trend line) were drawn. Then the antilogarithm of dose responsible for 50% of the response. (Yuji Nagawa et al 1971).

CONCLUSION

The median doses (ED_{50}) of both of them were calculated graphically and compared. The mean ED_{50} value of succinylcholine was found to be 1.59 ± 0.08µg (95% confidential limit was from 1.53 to 1.66µg). The ED_{50} of pancuronium was found to be 0.52 ± 0.10µg with 95% confidence limit being from 0.44 to 0.60µg. The ED_{50} value of the two drugs were very significantly different ($P < 0.001$). The potency ratio of pancuronium to succinylcholine was 0.32 i.e. succinylcholine is approximately 1/3 potent in comparison to pancuronium. The pancuronium appeared to be more potent than succinylcholine as found in this study conducted on frog rectus abdominis muscle.

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