EXPERIMENTS WITH BANANA TRUNK JUICE AS A NEUROMUSCULAR BLOCKER

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INTRODUCTION

The juice of the banana plant is used by some African tribes to poison their arrow tips (Heymer 1974). The wooden arrows are driven into the tree trunk for approximately 24 hr. Although Heymer did not name the species of banana used, it was decided to experiment with a local variety of Musa sapientum called pisang rajah.

The action of the fresh juice as well as a crude extract was studied and the results presented here.

PREPARATION

The trunk of the plant weighing approximately 2 kg was stripped and squeezed for its juice. Resin was allowed to separate out by coagulation and centrifugation. To prepare the extract, the juice (approximately 21) was freeze-dried to a residue of approximately 0.4 g.

METHOD

A cat was anaesthetised with chloralose (80 mg/kg) and pentobarbitone (10 mg/kg). An endotracheal tube was passed and was in position in case respiratory assistance was required. Blood pressure was recorded from a cannulated femoral artery through a Statham pressure transducer connected to a Grass Polygraph. Muscle contraction was recorded from the exposed tibialis anterior. The tendon of the muscle was tied with a string hooked to a force transducer which was connected up to the same polygraph.

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The anterior tibial artery was cannulated for retrograde intra-arterial injection of acetylcholine (ACh) and test substance. The brachial vein was cannulated for the injection of drugs other than ACh.

The peroneal nerve was exposed and stimulated electrically at 0.1 pulse per second, 0.1 or 0.2 millisecond duration and at supramaximal voltage.

An intra-arterial injection of ACh (10 ug) was carried out in the absence of electrical stimulation. After the blood pressure had stabilised, a dose of the test substance, (40 mg/ml in saline) was injected intra-arterially.

RESULTS

The first two experiments with juice showed a blockade which progressively became more severe until complete. Attempts to reverse it at this point with neostigmine and tetanic stimulation produced little results; Fig. 1.

Subsequent experiments were done using the extract reconstituted with saline.

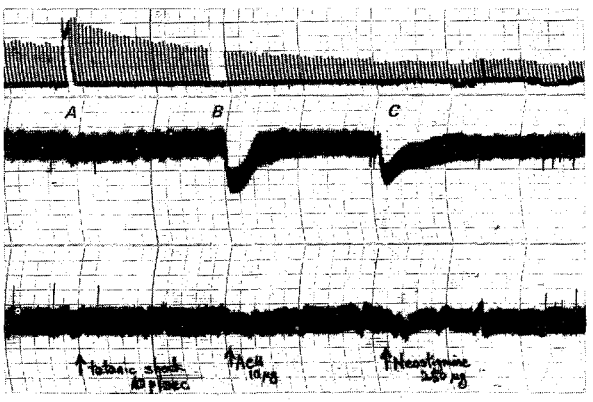

Fig 1. After a total dose of about 1 ml of the juice, at 'A', tetanic shock; at 'B', ACh I.A. and at 'C', Neostigmine I.V.

The first two of these showed slow onset and prolonged progression of block, as shown in Fig. 2. A decision was made to oxygenate the reconstituted extract before use. This resulted in a block, the onset of which was at about 10 min with a dose of 4 mg/kg intra-arterially:50% blockade was achieved in about 20 min (Fig. 3).

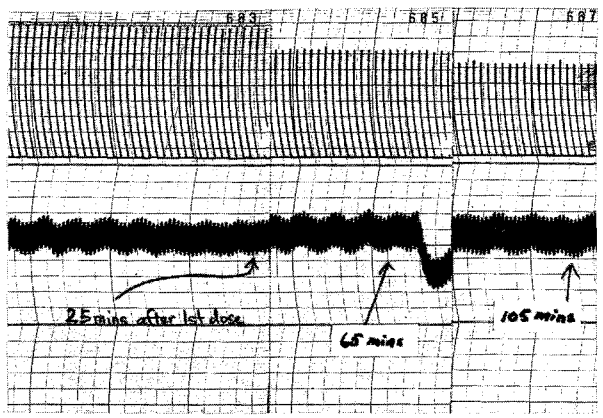

Fig. 2. Unoxygenated extract causes a slow and lesser blockade.

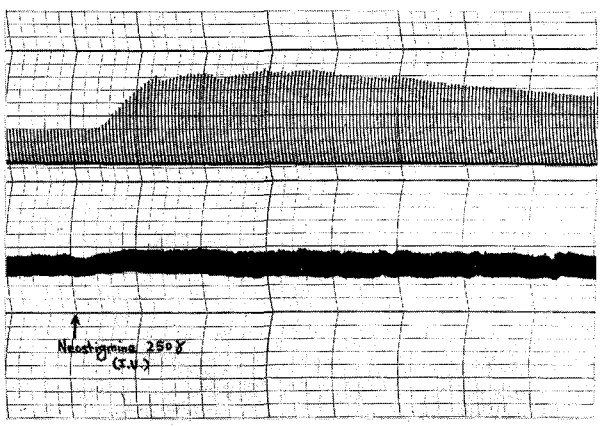

Fig. 3 A similar dose of oxygenated banana trunk extract produces a significantly faster onset of blockade. 50% block is achieved in 17 minutes.

Tetanic stimulation at 40 pulse per second produced a slight rise in the contraction height and neostigmine (250 ug I.V.) reversed the block. This reversal, as can be seen in Fig. 4, was transient and blockade returned in about 5 min without the progression being checked, that is, the gradient of the block was the same as before neostigmine. Inspite of repeated neostigmine and physostigmine injections, the progress of blockade was not arrested.

When the dose of the extract was reduced to 3 mg/kg and of neostigmine increased to 500 ug I.V., the outcome was the same as before (Fig. 5). No recovery was noticed even after three and one half hours of blockade.

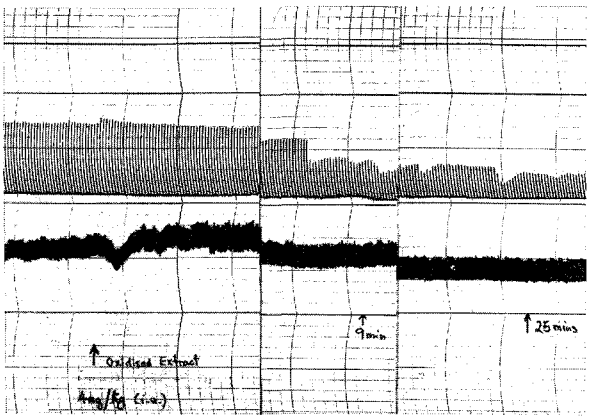

Fig. 4 Reversal of block by 250 ug of neostigmine is shown to be transient.

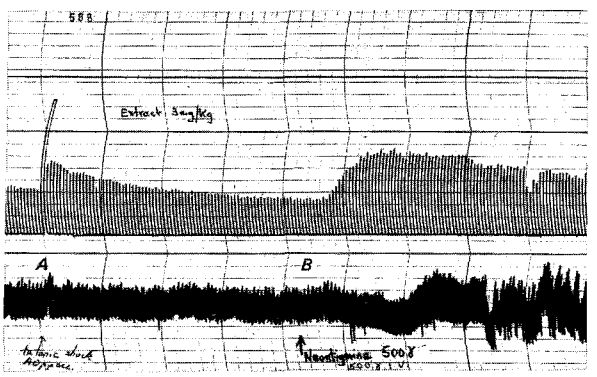

Fig. 5. 50% block is achieved after 50 minutes with a lower dose of extract. Full reversal with neostigmine is shown to be transient. At 'A', tetanic stimulation at 40 pps; at 'B', neostigmine 500 ug I.V.

DISCUSSION

In a non-depolarising blockade of the cholinergic receptor sites in skeletal muscle, the antagonist takes up the sites which ACh would normally occupy and therefore ACh cannot depolarise the muscle and produce the contractions. One way of reversal of such blocks is the use of anti-cholinesterases which allows a build-up of ACh.

The banana trunk extract appears to block in a way where excess ACh fails to reverse it. It was thought that the amount of active compound at the end plate region was sufficient to block again after the effects of neostigmine wore off. Lesser dosage has demonstrated it might not be so.

Release of ACh depends on many factors including calcium ions. Hubbard (1961) experimented with the rat diaphragm in a calcium-free bath and found that for increasing levels of calcium, the motor end plate potential frequency increased linearly to the log of calcium concentration in the bath. Elmqvist and Feldman (1965) also concluded that calcium is a necessary co-factor for the spontaneous release of ACh.

Compounds resembling curare in their mode of action did not affect the calcium dependence of ACh release as demonstrated by del Castillo and Stark (1952). This compound in the extract may differ from the curare type of compounds. It could also be binding to the calcium ions and affecting less ACh release.

Also probable is irreversible blockade as shown in effects of alpha-bungarotoxin (∝CBuTX). The effects of ∝C-BuTX have been demonstrated by Lee *et al.* (1977).∝C-BuTX produces long lasting non-depolarising neuromuscular block by irreversible binding to the receptors. Neostigmine produces short-lived antagonism of the block, the progression of the block not being arrested. Lee and Tseng (1966) used radioiodinated ∝C-BuTX and confirmed that toxin localised at the end plate zone. However, side effects of salivation and urination seen in our cats were not reported in experiments with ∝C-BuTX. No EKG abnormalities were noticed.

OBSERVATIONS

Whenever a dose of the extract or the fresh juice was injected, the height of contraction increased briefly with a drop in blood pressure.

It was tested for anticholinesterase activity and found to be a mild inhibitor of cholinesterase especially of plasma cholinesterase. This was done on human RBC and plasma after the method of Ellman *et al.* (1961) and modified by Ganendran (1974).

Blockade in rat diaphragm preparations showed partial reversal after washing.

SUMMARY

The juice of the banana trunk produces a non-depolarising neuromuscular block. Oxygenation of the extract enhances its potency. Reversal with anti-cholinesterase is transient.

Partial reversals in isolated preparations indicate there could be both specific and non-specific binding which could account for blockade after washing.

It could be specifically bound to ACh receptors in an irreversible way. Its action appears similar to that of ∝C-BuTX from the venom of the banded krait.

Purification of the extract and subsequent investigations will support present findings and present the characteristics fully.

REFERENCES

del Castillo, J. and Stark, L. (1952): The effects of calcium ions on the motor end plate potentials. *J. Physiol.* (Lond.), **116**: 507.

Ellman, G.L., Courtney, K.D., Andres, V. Jr. and Featherstone, R.M. (1961). A new and rapid colorimetric determination of Acetylcholinesterase activity. *Biochem. Pharmacol.*, 7: 88.

Elmqvist, D. and Feldman, D.S. (1965). Calcium dependence of spontaneous Acetylcholine release at mammalian motor nerve terminal. *J. Physiol. (Lond.)*, **181**: 487.

Ganendran, A. (1974). Pesticide (Organophosphate) intoxication in the human and the reactivation of phosphorylated acetylcholinesterase. Dissertation submitted to Bristol University, Bristol for M.D. degree.

Heymer, A. (1974). The hunting and food gathering Pygmies. *Image Roche*, **62**: 17.

Hubbard, J.I. (1961). The effect of calcium and magnesium on the spontaneous release of transmitter from mammalian motor nerve endings. *J. Physiol.* (lond.), **159**: 507.

Lee, C., Chen, D. and Katz, R.L. (1977). Characteristics of non-depolarising neuromuscular block: (1) Postjunctional block by alpha-bungarotoxin. *Canad. Anaesthe. Soc. J.*, **24**: 212.

Lee, C.Y. and Tseng, L.F. (1966). Distribution of bungarus multicinctus venom following envenomation. *Toxicon*, **3**: 281.