



## THE EFFECT OF NIFEDIPINE INDUCED CALCIUM ANTAGONISM ON IN VITRO TOXICITY OF LINDANE

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### ABSTRACT

The study of the effect of calcium antagonist (nifedipine) on isolated rabbit ileum poisoned with lindane was conducted. Rabbits of 1.2kg average body weight which was obtained from the Department of Pharmacology, Ahmadu Bello University, Zaria animal house were used for the current study. Each rat was sacrificed by a blow on the head, dislocation of the neck and exsanguinations. The abdomen was opened up and 4cm in length segment of the ileum obtained and mounted in an isolated organ bath containing Tyrodes solution. The preparation was maintained at 37°C aerated with air. (Kitchen, 1984; Dede, *et al*, 1991). 2 µg/ml lindane attenuated acetylcholine (1µg/ml) response, significantly different from acetylcholine response alone.  $P < 0.05$ ,  $P < 0.01$  in normal Tyrode's solution and  $Ca^{2+}$  free Tyrode's solutions respectively. 5µg/ml nifedipine attenuated acetylcholine response significantly different from acetylcholine response alone ( $P < 0.05$  in normal Tyrode's solution and  $Ca^{2+}$  free Tyrode's solution respectively. 5µg/ml nifedipine augmented reduction of acetylcholine response with 2µg/ml lindane significantly different from acetylcholine response alone  $P < 0.01$ , and  $P < 0.001$  in normal Tyrode's solution and  $Ca^{2+}$  free Tyrode's solution respectively. It was concluded that nifedipine (calcium channel blocker) augmented inhibitory action of lindane on acetylcholine response.

### INTRODUCTION

Drugs classified as  $Ca^{2+}$  entry blockers" belong to a group of compounds which inhibit calcium ion influx, hence lowering intra-cellular calcium ion concentration. This results in relaxation of the muscle (Ebeigbe, a & b; Marcus, 2002).

Reports by Fleckenstein, 1977 gave the concept that drugs could alter cardiac and smooth muscle contraction by blocking the entry of  $Ca^{2+}$  ion into myocytes.

Reports (Godfraind, 1982; Godfraind *et al*, 1986) have elucidated actions of nifedipine on calcium influx and isolated contraction of arteries. Furthermore, elucidation of calcium antagonism and classification of calcium channel blockers have been reported (David *et al*, 2002): these include:

- Phenylalkylamines e.g. verapamil
- Benzothiazepins e.g. Diltiazem
- Dihydropyridines e.g. Nifedipine, and nisoldipine
- Diarylaminoethylamine ether e.g. Bepridil
- Diphenylpiperazines

Lindane is a gamma hexachlorocyclohexane isomer of Benzene hexachloride a sub class of

organochlorines. Organochlorides are insecticides which are also found to be toxic to non-target mammalian tissues (Hassal, 1987; Iyaniwura *et al*, 1991; Dede *et al*, 1991; Dede & Dogara, 2004). Reports (Dede, *et al*, 1991; Hassal, 1987) have previously implicated calcium ions in the mechanism of action of lindane. Hassal (1987) further reported that  $ATP_{ase}$  dependent calcium and  $ATP_{ase}$  dependent  $Ca^{2+}/Mg^{2+}$  were sensitive to organochlorines.

The study further demonstrated that the inhibition of calcium  $ATP_{ase}$  resulted in destabilization of the membrane causing excitation. Furthermore, Dede *et al*, 1991 reported that increase in concentration of  $Ca^{2+}$  from 0.2% to 0.4% organ bath concentration, reversed inhibitory effect of lindane at the muscarinic receptor. These workers further reported that increased  $Mg^{2+}$  ion concentration in the organ bath augmented lindane effect.

Furthermore, two ion channels had been reported to admit calcium ions into the smooth muscle cell (Ebeigbe, 1987 a & b). These channels were potential sensitive channel (PSC) and Receptor Operated Channel (ROC). Activation of the receptor by such drugs as acetylcholine will culminate in influx of  $Ca^{2+}$  through the ROC and release of  $Ca^{2+}$  from the sarcoplasmic reticulum (SR). A rise in the intracellular  $Ca^{2+}$  will activate the contractile protein (Actin and Myosin) resulting in muscle

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contraction. The action of the calcium entry blockers is mediated through a selective inhibition of  $\text{Ca}^{2+}$  entry through voltage dependent channel (PSC) (Fleckenstein, 1977; Godfraind, 1982; Godfraind *et al*, 1986; Ebeigbe, 1987b).

At therapeutic concentrations,  $\text{Ca}^{2+}$  entry blockers had no effect on intracellular mobilization or movements of  $\text{Ca}^{2+}$  ion (Nayler and Poole – Wilson, 1981). However, report (Raliwani, *et al*, 1979) had extended the use of the term  $\text{Ca}^{2+}$  antagonists to include compounds which act intracellularly to interfere with  $\text{Ca}^{2+}$  supply. Walus *et al*, 1981 reported that  $\text{Ca}^{2+}$  entry blockers interfered with the release of  $\text{Ca}^{2+}$  from intracellular sites as well as with the influx through calcium channel.

Furthermore, reports (Bowstram *et al*, 1981; Johnson *et al*, 1982) had shown effects of  $\text{Ca}^{2+}$  channel blockers on calmodulin. In another report (Saida and Van Breemen, 1983), high concentration of diltiazem and nisoldipine had been shown to inhibit intracellular calcium from canine myentric artery.

## MATERIALS AND METHODS

Microdynamometer 7050 recorder (Ugo Basile, Milan, Italy) chemicals of analytical grade were obtained from the following sources:

Lindane 20% w/v (Gammalin 20<sup>R</sup>) imperial Chemical Industries, England. Nifedipine (Bayer Pharmaceutical, New Jersey, USA).

The Physiological solution used was Tyrode's solution 10 litres Tyrode's solution was prepared using the following sequence based on Kitchen, 1984.

Sodium Chloride, 90g; Potassium Chloride (10%), 20ml Sodium Biphosphate (10%); 5ml D-glucose; 10g Sodium bicarbonate; 10g Calcium Chloride (10%) 20ml and Magnesium Chloride 1ml. The solution was therefore made up to 10 litres with distilled water.

Rabbits weighing 1.2kg average body weight obtained from the Department of Pharmacology animal house were used for the current study. Each rabbit was sacrificed by a blow on the head, dislocating the neck and exsanguinations. The abdomen was opened up and the caecum exposed. The ileum was removed and the caecal end identified and cut into isolated segments (4cm in length). Each segment was mounted in the organ bath containing Tyrode's solution at 37°C, aerated with air (Kitchen, 1984; Dede *et al*, 1991; Iyaniwura *et al*, 1992).

The tissue was suspended in the organ bath for at least 30 min (equilibration period). Dose responses of the rabbit ileum 1µg/ml acetylcholine

was carried out. The tissue was then washed and allowed to rest. 5µg/ml nifedipine was added and 2min. antagonist contact time was allowed, without washing the tissue. 1µg/ml acetylcholine was added. The effect of nifedipine on acetylcholine response using normal Tyrode's solution was observed. The effect of 2µg/ml lindane on 1µg/ml acetylcholine was also carried out using the same procedure.

Furthermore, the physiological solution was changed to a  $\text{Ca}^{2+}$  free Tyrode's solution and the procedure was repeated. In each experiment, n = 5 and results reported  $\pm$  SEM. The results were further subjected to statistical evaluation using the student's t-test. ( $P < 0.05$  taken to be statistically significant).

## RESULTS

1µg/ml acetylcholine gave  $67.0 \pm 0.3\text{cm}$  and  $46.6 \pm 0.9\text{cm}$  responses (in normal and  $\text{Ca}^{2+}$  free Tyrode's solutions respectively).

2µg/ml Lindane attenuated the responses to acetylcholine (1µg/ml) both in normal and  $\text{Ca}^{2+}$  free Tyrode's solution. 5µg/ml nifedipine antagonized the response to acetylcholine ( $67.6 \pm 0.3\text{mm}$  to  $9.6 \pm 0.5\text{mm}$ ). Similar effect was obtained with  $\text{Ca}^{2+}$  free Tyrode's solution.

Nifedipine significantly augmented the response to lindane both with normal and  $\text{Ca}^{2+}$  free Tyrode's solution ( $P < 0.01$ ); ( $P < 0.001$ ) respectively (Fig. 2).

## DISCUSSION

Results obtained from the current study showed that the response to acetylcholine was attenuated by lindane and nifedipine respectively both in normal and  $\text{Ca}^{2+}$  free Tyrode's solution. This was in agreement with report by Dede *et al*, 1991 which indicated that lindane significantly attenuated the potentiation of acetylcholine by dichlorvos.

Also Iyaniwura *et al*; 1991 showed that interaction between dichlorvos and lindane at the muscarinic receptor resulted in attenuation of dichlorvos potentiation of acetylcholine response.

The mechanism of action of lindane had been associated with  $\text{Ca}^{2+}$  ions (Hassal, 1987; Dede *et al*, 1991). The reports indicated that lindane inhibited influx of calcium into the nerve membrane and muscles respectively. Considering events in contraction – excitation coupling of the muscle, depolarization of the plasma membrane resulted in electrical disturbance acting along the transverse tubule. This triggered  $\text{Ca}^{2+}$  release from triadic

junction (Bowman and Rand, 1980)  $Ca^{2+}$  would then be released from the vesicles of sarcoplasmic reticulum and bound to the troponin/tropomyosin actin complex, the existing repression of contraction is released. The resultant effect is activation of myosin  $ATP_{ase}$  and hydrolysis of ATP. Formation of cross bridges between actin and myosin and interdigitation of filament results in muscle contraction. After muscle contraction, sarcoplasmic reticulum –  $ATP_{ase}$  hydrolysis, ATP and  $Ca^{2+}$  is actively sequestered. Bowman and Rand, 1980 showed that  $Ca^{2+}$  free troponin/tropomyosin complex in conjunction with  $Mg^{2+}$  and intact  $ATP_{ase}$  inhibit formation of the cross bridge.

The site of action of  $Ca^{2+}$  channel blockers had been reported as the potential sensitive channel (PSC) and inhibition of release of  $Ca^{2+}$  from the sarcoplasmic reticulum (Ebeigbe, 1987a, b; Walus, *et al*, 1981; Saida and Van – Breman, 1981). From the results obtained, it could be explained that though nifedipine did not act at the receptor operated channel (Fig. 1) yet it inhibited acetylcholine by inhibition of the ultimate action of acetylcholine which was the release of  $Ca^{2+}$  from the sarcoplasmic reticulum.

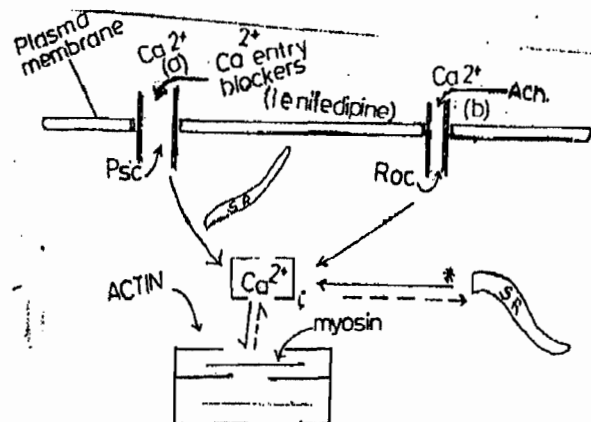
This seemed to be the factor responsible for the antagonism of acetylcholine response by nifedipine. Furthermore, lindane had been reported to inhibit  $Ca^{2+}$  influx into the nerve membrane (Hassal, 1987) and the muscle postsynaptic membrane (Dede, *et al*, 1991). The inhibition of  $Ca^{2+}$  influx by lindane could be responsible for the attenuation of acetylcholine response by lindane.

In the current study, nifedipine and lindane caused inhibition of  $Ca^{2+}$  entry into the muscle possibly at different sites but both agents showed complementary effects to one another. This was therefore responsible for the augmentation of inhibitory effect of lindane by nifedipine.

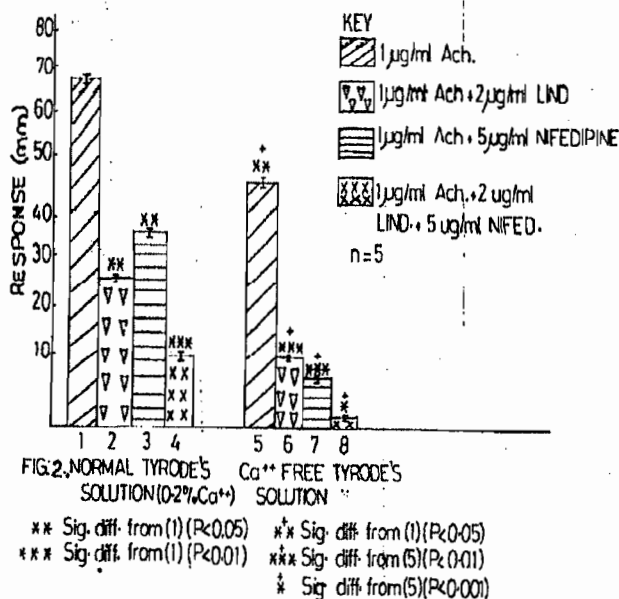
From the result obtained with  $Ca^{2+}$  free Tyrode's solution in which nifedipine and lindane individually and in combination attenuated responses due to acetylcholine, further suggested intracellular involvement of nifedipine and lindane in  $Ca^{2+}$  release possibly from the sarcoplasmic reticulum. The data suggested that the effect of lindane was calcium dependent, since it was mimicked by nifedipine a classical calcium channel antagonist.

Moreover, nifedipine, augmented the response to lindane. It was therefore, concluded that lindane acted through the calcium channel and inhibited the receptor activation response coupling by acetylcholine. The net effect was an attenuation of contraction of the smooth muscle by

lindane, action which was augmented by nifedipine via  $Ca^{2+}$  channel blockade at the potential sensitive channel (PSC) (Fig. 1).



**Fig. 1:** An illustration of events which occur with regard to  $Ca^{2+}$  influx at the smooth muscle. (Ach = acetylcholine). Two ion channels: Potential sensitive channel (PSC) and Receptor Operated Channel (ROC) admit  $Ca^{2+}$  into smooth muscle cell. In addition, receptor activation releases  $Ca^{2+}$  (asterisk) from intracellular stores sarcoplasmic reticulum (SR) (Adapted from Egbeigbe, 1987a, b).



**Fig. 2:** Effects of nifedipine, Calcium free Tyrode's solution on inhibitory response due to lindane n = 5,  $\bar{x} \pm Sem$

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