

## ORIGINAL RESEARCH ARTICLE

**Capsaicin Effect on Skeletal Muscle Nicotinic Receptor: A Preliminary Study.****Bhuvaneshwari S<sup>1\*</sup>, Nithya V<sup>2</sup>, Geetha V. Shastri<sup>3</sup>, Alice Kuruvilla<sup>4</sup>**

<sup>1</sup>Professor, Department of Pharmacology, <sup>2</sup>Former MBBS Student, <sup>3</sup>Former Professor, Department of Pharmacology, <sup>4</sup>Former Professor & HOD, Department of Pharmacology, PSG IMS &R, Peelamedu, Coimbatore-641004, Tamilnadu, India

Received 25 Sep 2013; Revised 15 Dec 2013; Accepted 26 Dec 2013

**ABSTRACT**

Capsaicin is the predominant chemical entity in the fruit of various species of Capsicum Solanaceae, commonly called Chili. Effect of capsaicin on nicotinic acetylcholine receptors on capsaicin sensitive nerves (Nn R) has been reported. But no such study has been reported so far for its effect on skeletal muscle nicotinic receptors (Nm R). Hence this study was undertaken to fill up this lacuna, by ascertaining its effect on skeletal muscle nicotinic receptors as agonist, potentiating agent or antagonist. A graded dose response curve with Ach in the presence and in the absence of Capsaicin were recorded and plotted. The results were plotted as log dose response curves and analysed for agonistic activity by Capsaicin. It was concluded that Capsaicin does not have direct action on skeletal muscle. It is also not an agonist of skeletal muscle nicotinic receptors. But it was potentiating the action of ACh on Nm Receptors. The vehicle Methanol also potentiated the effect of Ach to a lesser degree.

**Key words:** Capsaicin, Nicotinic receptors, skeletal muscle.**INTRODUCTION**

Capsaicin is the predominant chemical entity in the fruit of various species of Capsicum Solanaceae, commonly called Chili. It is a pungent alkaloid responsible for the characteristic sensation produced when chilies come in contact with mucous membranes. It is currently used locally or topically in treating painful conditions like cluster headaches, reflex sympathetic dystrophy, post-mastectomy pain, post-herpetic neuralgia and diabetic neuropathy<sup>[1]</sup>. Most of the current research on capsaicin is centered around the effect of capsaicin on pain mechanism. Some Preclinical and Clinical studies also have been conducted and reported on capsaicin's effect on other aspects as well. These include physiological functions like circulatory, gastrointestinal, psychological, behavioral, immunological and metabolic functions and as well as its pro and anti carcinogenic and anti infective potential<sup>[2]</sup>. Effect of capsaicin on nicotinic acetylcholine receptors on capsaicin sensitive nerves (Nn R) has been reported<sup>3</sup>. But no such study has been reported so far for its effect on skeletal muscle nicotinic receptors (Nm R). Hence this study was undertaken to fill up this lacuna, by ascertaining

its effect on skeletal muscle nicotinic receptors as agonist, potentiating agent or antagonist.

**MATERIALS AND METHODS****Preparation of Solutions:**

The drugs like Capsaicin, Acetylcholine and Pancuronium were procured from Sigma Chemical while other chemicals were from Loba. Molar solutions of Ach and Pancuronium were prepared using water as vehicle while that of Capsaicin, a non-aqueous soluble compound, was prepared using methanol as vehicle. In order to minimize the influence if any of methanol 100% and 50% methanol were used as vehicle. Appropriate dilutions of each drug using the vehicle was prepared using dilution method for testing.

**Biological material and experimental procedures:**

The experimental set up was done using the standard technique for isolated rectus abdominis muscle preparations from frog<sup>[4]</sup>.

**Study Groups:**

Three groups (10 per group) consisting of

1. **Group Ach:** Ach alone (to compare the

effect with Capsaicin)

2. **Group Ach + M + C:** Ach + Capsaicin in 50% and in 100% methanol (to assess antagonistic/potentiating activity)
3. **Group Ach + M:** Ach + 50% & 100% methanol (to rule out or take into account vehicle effect)

### Recording the effect on nicotinic skeletal muscle:

The effect on skeletal muscle was recorded using student physiograph.

### Method for assessment of Nicotinic Receptor Stimulation:

A graded dose response curve with Ach alone and Capsaicin alone was obtained using suitable dilution and increments of dose. The results were plotted as log dose response curves and analysed for agonistic activity by Capsaicin.

### Assessment as to Potentiation or Blockade of Ach effect by Capsaicin:

The graded dose response curves for Ach in the presence and in the absence of Capsaicin were recorded and plotted. Shift of log dose curve of Ach to right by Capsaicin was considered as blocking effect, while shift to left was considered as potentiating effect.

### Quantification of Capsaicin effect:

This was done using appropriate formula as outlined by MN Gosh<sup>5</sup> after calculation of ED 50.

### Analysis of results:

The results were analysed using Student paired 't' test.

### Ethics:

Study was conducted after Institutional Animal Ethics committee approval. And we have adhered to the guidelines of our institution regarding the care and use of laboratory animals.

## RESULTS

### Effect of Capsaicin alone:

Capsaicin alone up to 1mM dose did not produce response ( $p < 0.001$ ) compared to Ach when tested on the same tissue preparation (**Table 1**).

### Combined effect of Ach and Capsaicin/Methanol:

A typical 'set of responses' was obtained for a single dose of Ach alone, Capsaicin in 50% methanol administered 90 seconds prior to Ach administration and 50% methanol administered 90 seconds prior to Ach administration (**Figure 1**). The combined administration of Ach and Capsaicin resulted in an increase in response to

the same dose of Ach. The vehicle methanol also potentiated the Ach action but to a less degree.

Similar sets of responses were obtained in the same preparation with sub maximal doses of Ach in a graded fashion (n: 10). The entire procedure was repeated using 100% methanol as vehicle (n: 10). The height of contractions produced for graded doses of Ach in the presence and absence of 50% and 100% methanol as well as in the presence and absence of Capsaicin in 50% and 100% methanol were tabulated (**Table 2**). These results were plotted as log dose response curves (LDRCs) of Ach (**Figure 2**).

## DISCUSSION

Capsaicin alone up to 1mM dose did not produce response ( $< 0.001$ ) compared to Ach when tested on the same tissue preparation. This indicates that Capsaicin as such has no agonistic activity on Nm Receptors.

The combined administration of ACh and Capsaicin resulted in an increase in response to the same dose of Ach, pointing out that it did not block but potentiated the Ach action on Nm receptor. The vehicle methanol also potentiated the Ach action but to a less degree, indicating that Capsaicin, independent of methanol, has caused potentiation on its own.

Analysis of LDRC in Figure 2 shows that both the vehicle as well as test drug Capsaicin potentiate ACh effect as exemplified by shift of LDRC of ACh to left parallel, But the LDRCs in the presence of 100% Methanol and Capsaicin in 100% Methanol are overlapping whereas that using 50% methanol are fairly apart at least in lower doses.

Tissue preparations differ in their threshold for response due to biological variation and therefore the responses observed will become more meaningful when the above responses are converted into % of Maximum Probable Effect (%MPE) using the formula,

$\%MPE = 100 \times \frac{\text{Responses for a given dose on a tissue}}{\text{Maximal response by the same tissue}}$

**Table 1: Statistical analysis between groups**

Groups compared	100% Methanol	50% Methanol
Ach Vs M	$P < 0.001$	$P < 0.001$
Ach Vs C + M	$P < 0.001$	$P < 0.001$
M Vs C + M	$P > 0.05$	$P < 0.01$ ( 5- 40 $\mu$ g) $P > 0.05$ ( 80-106 $\mu$ g)

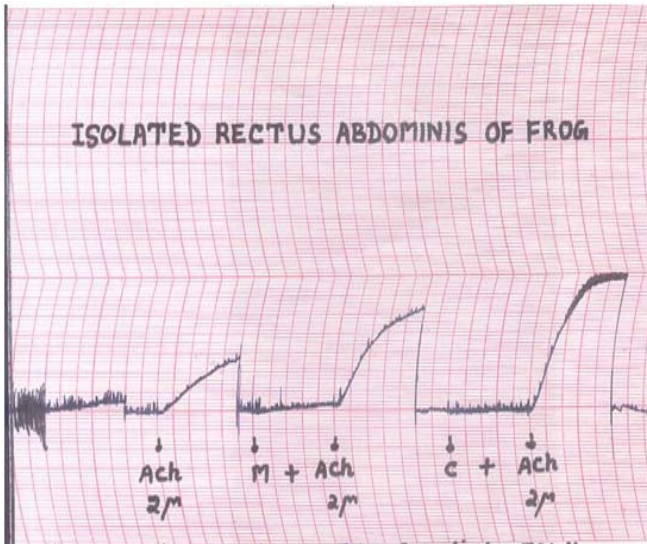
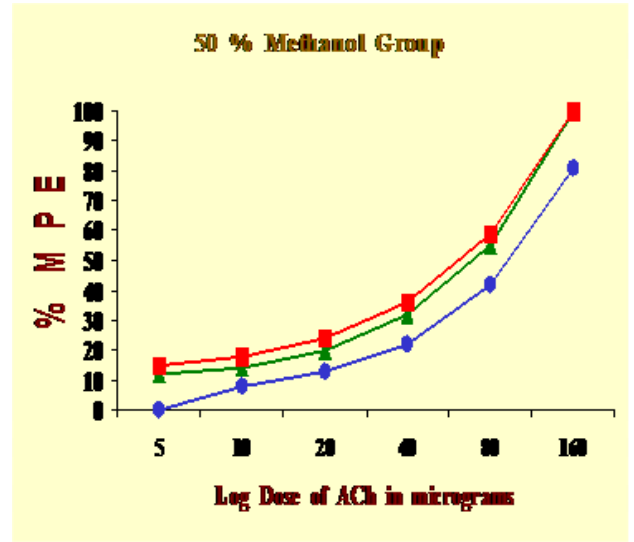


Figure 1: Response to Ach, Ach + M, Ach + M + C



■ ACh ■ M + Ach ■ C + M + Ach

Figure 3: % Maximum Probable Effect using 50% Methanol

(Table 3) gives the Mean  $\pm$  SD of % MPE of different groups. %MPE for a given dose of ACh was analysed statistically in the presence and absence of 50% or 100% Methanol alone or with Capsaicin, using Student paired 't' test. The results were tabulated in (Table 1). It was evident from this table that Capsaicin effect was statistically significant from that of the vehicle Methanol only when 50% Methanol was used and that too for Ach doses 5 to 40  $\mu$ M only. This indicates that undiluted vehicle masks the Capsaicin effect and this influence of the vehicles was minimized by 1:2 dilution of the vehicle with water. Further dilution of the vehicle was not possible as it resulted in turbid solution.

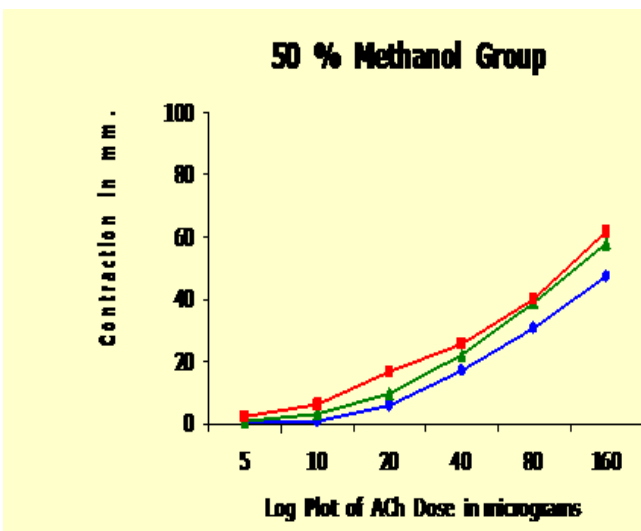
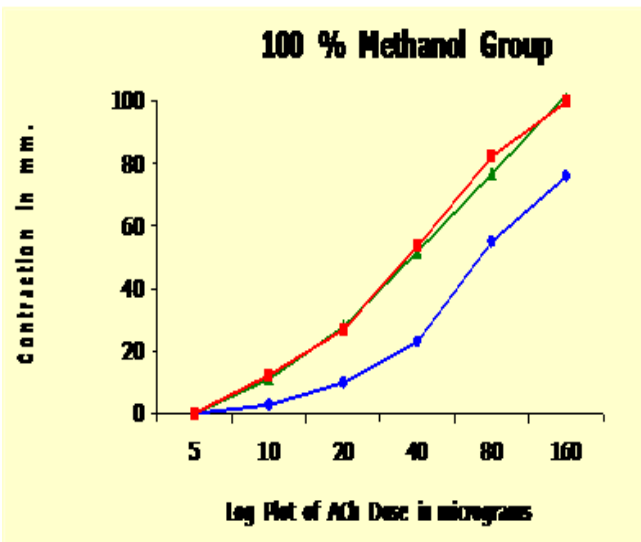
The potentiation by Capsaicin was calculated as follows:

Potentiation by Capsaicin in Methanol-  
Potentiation by Methanol alone

Potentiation was calculated using the formula<sup>6</sup> given below:

Potentiation = antilog mean {neg log ED 50(after) - neg log ED 50 (before)}

The ED 50 was calculated<sup>7</sup> by plotting the %MPE for all doses of ACh as Log dose response line (Regression line) using the statistical calculation for regression equation<sup>8</sup> (Figure 3). Potentiation by Capsaicin was thus quantified at different dose levels of ACh and tabulated (Table 4). It was obvious that potentiation was more marked at lower doses than at higher doses of ACh.



■ ACh ■ M + Ach ■ C + M + Ach

Figure 2: LDRCs OF ACh using 100% & 50% Methanol

Table 2: Mean  $\pm$  SD Actual Responses in mm

% of Methanol	Groups	Ach dose in $\mu$ M					
		5	10	20	40	80	160
50% Methanol	Ach	0.2 $\pm$ 0.2	0.7 $\pm$ 0.4	5.8 $\pm$ 2.3	17 $\pm$ 5.6	30.8 $\pm$ 9.8	47.3 $\pm$ 15.7
	Ach + M	0.9 $\pm$ 0.3	3.3 $\pm$ 1	9.8 $\pm$ 2.9	22.1 $\pm$ 6.3	38.8 $\pm$ 11.5	59.4 $\pm$ 19
	Ach+M+C	2.2 $\pm$ 0.9	6.3 $\pm$ 2.5	16.7 $\pm$ 6.0	25.6 $\pm$ 7.8	40 $\pm$ 12.8	62 $\pm$ 20.6

100 % Methanol	Ach	0	2.8±0.9	10±1.6	23±4.3	55±7.0	76±10
	Ach + M	0	12.2±2.0	26.9±4.9	53.4±8.4	82.3±9.0	99.8±11
	Ach+M+C	0	11.7±2.0	27.5±5.0	51.6±7.6	76.5±7.7	102±11

**Table 3: Mean ± SD % of Maximum Probable Effect**

% of Methanol	Groups	Ach dose in µM					
		5	10	20	40	80	160
50% Methanol	Ach	0	1.6±0.8	10.3±2.7	29.6±6.5	46.1±5.1	78.1±3.9
	Ach + M	1.5±0.5	6.2±1.2	18.9±3.4	42.4±6.1	67.8±9.3	89.5±11.6
	Ach+M+C	3.4±1.0	10.5±1.5	28.3±4.1	46.3±5.8	69.2±4.4	100±0.1
100 % Methanol	Ach	0	2.5±1.5	9.4±1.2	21.5±3.3	50.5±5.01	71.3±2
	Ach + M	0	9.0±1.6	22.9±2.6	46.8±4.3	72.3±4.7	94.6±2.3
	Ach+M+C	0	11.1±1.3	24.1±3.3	48.7±4.8	70.6±4.1	96.4±2.1

**Table 4: Potentiation by Capsaicin**

Dose levels of Ach	Potentiation by Capsaicin
ED 20	0.943
ED 30	0.544
ED 40	0.322
ED50	0.270

## REFERENCES

1. Winter J, Bevan S & Campbell E A. Capsaicin and Pain mechanisms. *Br J Anaesth* 1995; 75:157.
2. Institute for Natural Products Research Capsicum, Page 1-12.
3. Roberts R G, Stevenson J E, Westerman R A & Pennefather J E. Nicotinic acetylcholine receptors on capsaicin sensitive nerves. *Neruo Report Rapid Communications of Oxford Ltd. Oxford UK* 1995; 6:1578.
4. The Staff of the Department of Pharmacology-University of Ediburgh, Pharmaceutical Experiments on Isolated Preparations ( E & S. Livingstone Ltd. Edinburch and London) 1968:38.
5. Ghosh M N. Fundamentals of Experimental Pharmacology, 2<sup>nd</sup> Edition. (Scientific Book Agency, Calcutta) 1984:98-118.
6. Ghosh M N. Fundamentals of Experimental Pharmacology, 2<sup>nd</sup> Edition. (Scientific Book Agency, Calcutta) 1984:106.
7. Ghosh M N. Fundamentals of Experimental Pharmacology, 2<sup>nd</sup> Edition. (Scientific Book Agency, Calcutta) 1984:102.
8. Ghosh M N. Fundamentals of Experimental Pharmacology, 2<sup>nd</sup> Edition. (Scientific Book Agency, Calcutta) 1984:184.