Original Article

Influence of Selected Metal Ions on the Contraction Produced by Acetylcholine/ Nicotine on Rectus Abdominis Muscle

P. Veeresh Babu[*1], O.Palakshi Gouda[1], J. Rajasekhar[1], S. Satyanarayana[1], Shelby E. Brooks[2], Bettaiya Rajanna[2]

[1]College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India.
[2]Alcorn State University, Alcorn State, Mississippi, USA.

Address for Correspondence

Avanthi Institute of Pharmaceutical Sciences
Cherukupally (v),
Vizianagaram (Dist)
Pin: 531162
Phone: +919493530528
E-mail:
pratap.veeresh@gmail.com

ABSTRACT

Objective: To investigate the influence of selected metal ions $CaCl_2$, $BaCl_2$, $SrCl_2$, $ZnCl_2$, $CoCl_2$, $CuCl_2$ and $FeCl_3$ on the effect of acetylcholine on nicotinic receptors of skeletal muscle of the frog. Since nicotine also acts on nicotinic receptors, the influence of metal ions on nicotine was also studied.
Method: The dose-response curve of acetylcholine and nicotine was obtained by using isolated rectus abdominis muscle of frog. The influence of various concentrations of selected metal salt solutions (1-1600µg) was tested on selected submaximal response of acetylcholine and nicotine.
Results: $CaCl_2$ potentiated the effects of both acetylcholine and nicotine. But $BaCl_2$ and $SrCl_2$ improved acetylcholine activity without altering nicotine response whereas $CoCl_2$ and $ZnCl_2$ reduced nicotine action without modifying acetylcholine action. The ions of $CuCl_2$ and $FeCl_3$ did not alter the contraction produced by both acetylcholine and nicotine.
Conclusions: The study demonstrates that supplementation of $CaCl_2$, $BaCl_2$ and $SrCl_2$ may be useful in the case of myasthenia gravis and metal ions can be used to distinguish the acetylcholine action from that of nicotine at N_MR.

Keywords: Metal ions, Acetylcholine, Nicotine, Skeletal muscle.

INTRODUCTION

Many metals play critical roles in maintaining life. Some are important for the structure of biological systems including cell to organ, as calcium is for bone[1]. Some trace elements, despite their low concentrations, are of great importance for growth and development of living system. Hence they are termed as being essential if depletion consistently results in a deficiency syndrome and repletion specifically reverses the abnormalities[2]. Metals also serve important

role as essential components of many enzymes[3].

The use of metals in medicine traces back to antiquity with various elements, such as arsenic, gold and iron being used to treat different ailments. Healthy mammals require large number of bio-essential trace metals and their deviation from normal concentrations leads to disease. For example, deficiency of iron results in anemia[4].

Some neuromuscular disorders like myasthenia gravis suffers from inadequate acetylcholine at neuromuscular junction. Acetylcholine is completely dependent on intracellular and extracellular calcium for proper muscle contraction. Inadequate acetylcholine at neuromuscular junction leads to release of less calcium from sarcoplasmic reticulum. Deficiency of calcium results in inefficient muscle contraction. It leads to fatigue and lassitude, which are the main symptoms of myasthenia gravis (MG)[5]. It is the major skeletal muscle disorder of autoimmune nature, which is caused by generation of autoantibodies against the nicotinic acetylcholine receptors (nAChRs) on the postsynaptic membrane of the neuromuscular junction (NMJ)[6,7]. Inadequate acetylcholine[8,9] and lack of free nicotinic receptors at NMJ[10,11] are major etiologic factors of MG. ACh released from motor nerve endings acts on nicotinic receptors whereas at neuroeffector junctions acts on muscarinic receptors[12]. But in MG, nicotinic action of acetylcholine is essential rather than muscarinic action. Acetylcholinesterase (AChE) inhibitors, corticosteroids, immunosuppressants, thymectomy, short term interventions such as plasmapheresis and intravenous immunoglobulin(IVIg) are the treatment modalities available for MG[13]. Clinically methylcarbamates remain first line drugs for their well known AChE enzyme inhibitory action. But they possess severe peripheral muscarinic side effects such as gastrointestinal cramps, diarrhoea, increased lacrimal, salivary, and bronchial secretions, sweating, bradycardia and hypotension[14]. The other classes of drugs also possess severe complications.

Nicotine, an alkaloid that occurs in tobacco also produces contraction of skeletal muscle similar to acetylcholine. However nicotine cannot be used as a drug because it is well known for habit forming nature[15]. Structurally acetylcholine and nicotine are unrelated. So alternatives that work on the same receptors or improve acetylcholine activity at the same site selectively have to be developed**.** It was reported that barium has a strong predilection for muscle tissue, markedly increasing contractility[16]. Based on this report, it was planned to investigate the influence of selected metal ions such as $CaCl_2$, $BaCl_2$, $SrCl_2$, $ZnCl_2$, $CoCl_2$, $CuCl_2$ and $FeCl_3$ on the contraction produced by acetylcholine on rectus abdominis muscle of frog. Since nicotine has similar action, their influence on nicotine was also studied on the same muscle.

MATERIALS AND METHODS

Animals

Frogs, obtained from Ghosh Enterprises, Kolkata, were used in the study. All animal experiments were approved by the Institutional Animal Ethics Committee (registration no. 516/01/A/CPCSEA) and followed the guidelines of both the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and the International Guidelines for Handling of Laboratory Animals.

Chemicals

Acetylcholine HCl was procured from Sigma Aldrich. Nicotine was obtained as gift sample from Central Tobacco Research

Institute. Metal salts such as $CaCl_2$, $BaCl_2$, $SrCl_2$, $ZnCl_2$, $CoCl_2$, $CuCl_2$ and $FeCl_3$ were obtained from Merck Limited.

Solutions

Ringer solution was used as physiological salt solution, which consists of NaCl, KCl, $CaCl_2$, $NaHCo_3$ and glucose[17].

Equipment

Kymograph, Sherrington rotating drum, simple organ bath and simple writing lever were used for recording of muscle responses.

Procedure

Frog (*Rana tigrina*) was pithed by destroying the brain and spinal cord with the help of pithing needle. The skin on the abdomen was removed and the rectus abdominis muscle was exposed. A midline incision on the abdominal muscle was made from pelvic girdle to pectoral girdle[17]. The muscle was divided into two halves and pieces of threads were tied to both ends of one half of the muscle. It was detached from the body and mounted in up-right position in the organ bath containing frog Ringer solution with a tension of 0.5g.The organ bath was aerated and the tissue was stabilized for 45 min with fresh quantum of Ringer for four times. The contraction due to acetylcholine / nicotine was recorded with 30sec baseline, 90 sec contact time, 2min rest time with a total 5 min time per cycle[18]. Response to increasing doses of acetylcholine was recorded until ceiling response was observed. Its submaximal response was selected and reproducibility was tested. The influence of various concentrations of selected metal salt solutions (1 - 1600µg) was tested on submaximal response of acetylcholine. Similarly different concentrations of metal solutions were tested on selected submaximal response of nicotine.

Statistical test of significance

Data was reported as mean ± SEM. They were analyzed by Student's paired T-test.

RESULTS

The dose response curve of acetylcholine and nicotine was recorded. Sub-maximal response was selected and the influence of selected metal ions was evaluated.

Effect of metal ions on acetylcholine

The effect of metal ions was tested on acetylcholine in the dose ranging from 1µg-1600 µg. $CaCl_2$, $BaCl_2$ and $SrCl_2$ potentiated the activity of acetylcholine. The lower doses of $CaCl_2$ were unable to influence the acetylcholine. The potentiation was first observed at 64 µg which gradually enhanced with increase in dose. The maximum potentiation was seen at 128µg (Fig.1). Similar trend was seen in the case of $BaCl_2$ and $SrCl_2$.The minimum dose of $BaCl_2$ and $SrCl_2$ required to potentiate the acetylcholine activity was found to be 200 µg and 800 µg respectively. The maximum potentiation was observed at 400 µg of $BaCl_2$ and 1600 µg of $SrCl_2$ (Fig.2 & 3). In the above cases the salts have shown the dose dependent effect. The other metal ions like $ZnCl_2$, $CoCl_2$, $CuCl_2$ and $FeCl_3$ did not alter the response of acetylcholine (Table 1).

Effect of metal ions on nicotine

The influence of metal ions was evaluated on nicotine in the dose range of 1µg-1600 µg. $CaCl_2$ potentiated the effect of selected dose of nicotine. Initially the potentiation was observed at 128 µg and maximum potentiation was seen at 200 µg (Fig. 4). In contrast $CoCl_2$ and $ZnCl_2$ reduced the activity of nicotine. The reducing effect of $CoCl_2$ was initially observed at 400 µg. The reduction in nicotine activity was enhanced

with increase in the dose of $CoCl_2$. The maximum reduction was observed at 800 µg and thereafter it remained constant (Fig. 5). Similarly $ZnCl_2$ initially reduced the nicotine activity at 800 µg and maximum reduction was observed at 1600 µg (Fig. 6). Both $CoCl_2$ and $ZnCl_2$ have shown dose dependent reducing effect on nicotine. Other metal salts like $BaCl_2$, $SrCl_2$, $CuCl_2$ and $FeCl_3$ did not modify the contractile effect of nicotine (Table 2).

DISCUSSION

About 30 elements are now believed to be essential to life. They can be categorized as bulk elements-H, Na, Mg, K, Ca, C, N, O, P, S, Cl, essential trace elements-Li, V, Cr, Mo, Mn, Fe, Co, Ni, Cu, Zn, B, Si, As, Se, F, I and proposed essential trace elements-Cd, Sn, Pb. The physiological role of essential elements is well established[19].

Metals had been used in the treatment of different ailments. Diseases such as anaemia (iron), asthma (gold and magnesium), bipolar disorders involving alternative mania and depression (lithium), diabetes (vanadium), rheumatoid arthritis (gold), stroke (magnesium), tropical diseases (antimony and rhodium) and ulcers (bismuth) can be treated by either pure metals or their compounds. Nowadays cisplatin is widely used in the treatment of testicular tumors.

The deviation from normal concentrations of these metals leads to disorders. Deficiency of fluorine leads to dental caries, selenium results in liver necrosis, iron leads to anaemia, etc[20].

Some of the metal ions are involved in the transmission of neuromediator function. For example acetylcholine, released from motor nerve endings acts on nicotinic muscular receptors (N_MR) located on the motor end plate of skeletal muscle[21]. It induces conformational changes in the receptor proteins and opens the ion channels. Influx of sodium and calcium ions occurs resulting in depolarization of the muscle. This depolarization propagates deeply into the muscle fiber and causes the sarcoplasmic reticulum to release into the sarcoplasm large quantities of calcium ions. The released calcium ions initiates attractive forces between the actin and myosin filaments causing them slide together, resulting in muscle contraction in in-vivo conditions[22]. The present work demonstrates similar effect with added acetylcholine on an isolated (denervated) skeletal muscle mounted in the organ bath. Its effect was increased by $CaCl_2$, which is understandable since extracellular calcium substantially augments the membrane depolarization. It results in more release of calcium from sarcoplasmic reticulum and in turn increased contraction[23, 24]. The $CaCl_2$ also improved the nicotine action indicating that it is required in nicotine produced action also. However $BaCl_2$ and $SrCl_2$ improved acetylcholine activity without altering nicotine action. They might have improved acetylcholine activity by enhancing the membrane depolarization similar to $CaCl_2$.

On the other hand $CoCl_2$ and $ZnCl_2$ reduced nicotine action without altering acetylcholine activity. It was reported that $CoCl_2$[25] forms complex with nicotine at physiological pH and the same might be responsible for the reduction in nicotine action by decreasing the number of free nicotine molecules available for stimulation of the receptors. Similar phenomenon might be responsible for reduction in nicotine action in the presence of $ZnCl_2$. Hence there appears to be difference in between acetylcholine and nicotine action on N_M receptors.

The compounds $CuCl_2$ and $FeCl_3$ did not alter the activity of both acetylcholine and nicotine. Hence in in vivo condition it can be assumed that the presence of $CoCl_2$, $ZnCl_2$, $CuCl_2$ and $FeCl_3$ do not affect the contractile activity of acetylcholine on skeletal muscle.

CONCLUSION

$CaCl_2$ is one of the ions present in the body, which plays vital role in muscle contraction. In the case of myasthenia gravis calcium supplementation may help. Whether $BaCl_2$ and $SrCl_2$ can also be used as supplements is to be investigated further by determining their toxicities. The efficiency of metal ions ($CaCl_2$, $BaCl_2$ and $SrCl_2$) as supplements to therapy of myasthenia gravis needs in vivo evaluation to confirm their potentialities. The study demonstrates that certain metal ions ($BaCl_2$, $SrCl_2$, $CoCl_2$ and $ZnCl_2$) can be used to distinguish the action of acetylcholine and nicotine on the skeletal muscle.

ACKNOWLEDGEMENTS

Our special thanks are due to University Grants Commission (UGC), New Delhi, for awarding Junior Research Fellowship to carry out this work. The authors thanks the NIH/NCMHD/MHIRT GRANT#9 T37 MD001532-05, Alcorn State University, Mississippi, USA for providing financial assistance.

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Table 1. Effect of selected metal ions on acetylcholine contraction on rectus abdominis muscle of frog

Dose of the metal salt (µg)	Height of curve in mm (ACh response)						
	$CaCl_2$	$BaCl_2$	$SrCl_2$	$CoCl_2$	$ZnCl_2$	$CuCl_2$	$FeCl_3$
Control	49.84±0.9	41.28±0.3	44.12±0.6	46.01±0.7	45.98±0.52	48.34±0.64	53.96±0.48
1	49.67±0.4	41.33±0.6	44.67±0.4	45.67±0.3	46.00±0.31	48.00±0.73	54.00±0.73
2	48.67±0.6	41.33±0.9	45.33±0.3	46.00±0.8	45.33±0.33	48.33±0.30	53.00±0.68
4	49.00±0.3	41.67±0.6	46.33±0.3	46.00±0.6	44.67±0.91	48.67±0.40	53.33±0.40
8	49.00±0.7	42.33±0.7	45.00±0.5	46.00±0.3	45.33±0.60	48.00±0.75	53.67±0.76
16	48.67±0.3	41.33±0.7	44.33±0.4	46.00±0.4	44.67±0.33	47.33±0.88	53.00±0.68
32	48.00±0.7	41.33±0.1	44.67±0.4	45.67±0.7	45.67±0.45	46.33±0.29	54.00±0.75
64	54.33±0.7	41.33±0.8	43.33±0.3	45.33±0.4	46.33±0.30	45.67±0.45	54.67±0.45
128	58.00±0.5	42.00±0.5	43.33±0.4	44.67±0.6	47.00±0.68	47.00±0.73	54.33±0.67
200	57.00±0.5	49.67±0.7	44.33±0.2	45.33±0.8	47.33±0.33	47.67±0.83	54.33±0.67
400	-	57.00±0.7	45.00±0.4	46.00±0.6	47.00±0.28	48.67±0.83	55.00±0.58
800	57.64±0.7	58.33±0.4	52.33±0.3	45.67±0.4	46.00±0.86	49.33±0.45	55.00±0.73
1600	57.94±0.5	57.83±0.4	56.67±0.8	45.33±0.4	46.00±0.59	48.33±0.45	54.67±0.76

n = 6, Significant at P<0.01**, P<0.001***

Table legend: The effect of selected metal ions on acetylcholine produced muscle contraction was described in this table. $CaCl_2$, $BaCl_2$ and $SrCl_2$ potentiated the effect of acetylcholine while $CoCl_2$, $ZnCl_2$, $CuCl_2$ and $FeCl_3$ did not alter the effect of acetylcholine.

µg - micrograms; mm - millimeters.

Table 2. Effect of selected metal ions on nicotine contraction on rectus abdominis muscle of frog

Dose of the metal salt (µg)	Height of curve in mm (Nicotine response)						
	$CaCl_2$	$BaCl_2$	$SrCl_2$	$CoCl_2$	$ZnCl_2$	$CuCl_2$	$FeCl_3$
Control	40.45±0.6	44.64±0.4	44.08±0.8	41.29±0.4	52.68±0.68	55.92±0.52	55.98±0.36
1	40.63±0.3	44.28±0.6	44.27±0.2	41.69±0.3	52.47±0.52	55.62±0.27	55.81±0.91
2	40.93±0.7	44.42±0.9	44.30±0.8	41.54±0.4	52.62±0.13	55.11±0.32	55.49±0.42
4	40.00±0.5	44.67±0.3	44.33±0.6	41.00±0.5	52.00±0.76	55.00±0.53	55.33±0.33
8	40.33±0.4	45.67±0.2	45.00±0.8	41.00±0.7	52.67±0.66	55.33±0.40	55.33±0.40
16	39.00±0.5	46.00±0.4	44.67±0.4	40.67±0.8	53.00±0.54	55.33±0.60	55.00±0.58
32	40.00±0.5	45.33±0.9	45.00±0.5	40.67±0.3	53.33±0.33	56.33±0.60	55.67±0.40
64	40.33±0.3	45.33±0.4	44.00±0.5	41.33±0.8	52.67±0.67	55.67±0.43	56.33±0.33
128	44.67±0.8	44.67±0.9	42.67±0.6	42.33±0.8	51.67±0.67	56.33±0.91	57.00±0.68
200	47.67±0.8	43.67±0.9	43.67±0.4	42.33±0.8	52.33±0.37	55.67±0.45	56.67±0.79
400	47.00±0.5	44.67±0.9	43.67±0.8	33.00±0.5	52.33±0.48	55.00±0.88	57.67±0.49
800	47.62±0.4	44.67±0.6	44.00±0.5	27.33±0.8	43.33±0.37*	54.67±0.40	56.67±0.33
1600	47.34±0.7	44.92±0.7	44.00±0.6	27.33±0.4	38.33±0.37*	55.00±0.53	56.00±0.65

n = 6, Significant at P<0.01**, P<0.001***

Table legend: The effect of selected metal ions on nicotine produced muscle contraction was described in this table. $CaCl_2$ potentiated the effect of nicotine but $CoCl_2$ and $ZnCl_2$ reduced its effect. while $CuCl_2$ and $FeCl_3$ did not alter the effect of nicotine.

µg - micrograms; mm - millimeters.

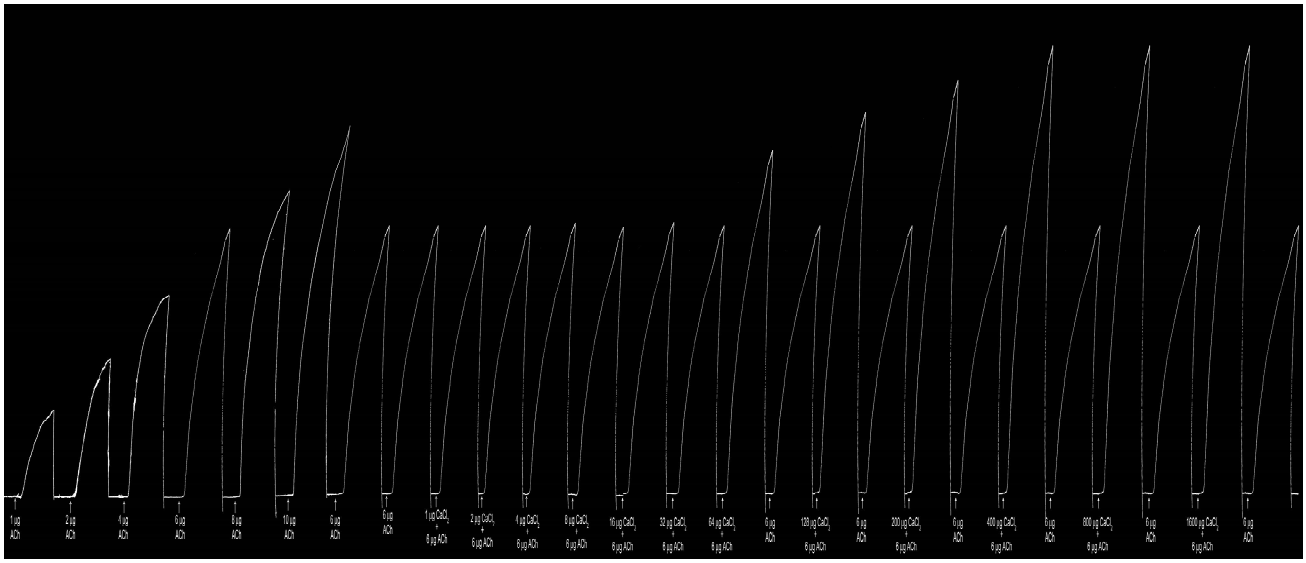

Figure.1. Effect of $CaCl_2$ on skeletal muscle contraction produced by acetylcholine in frog

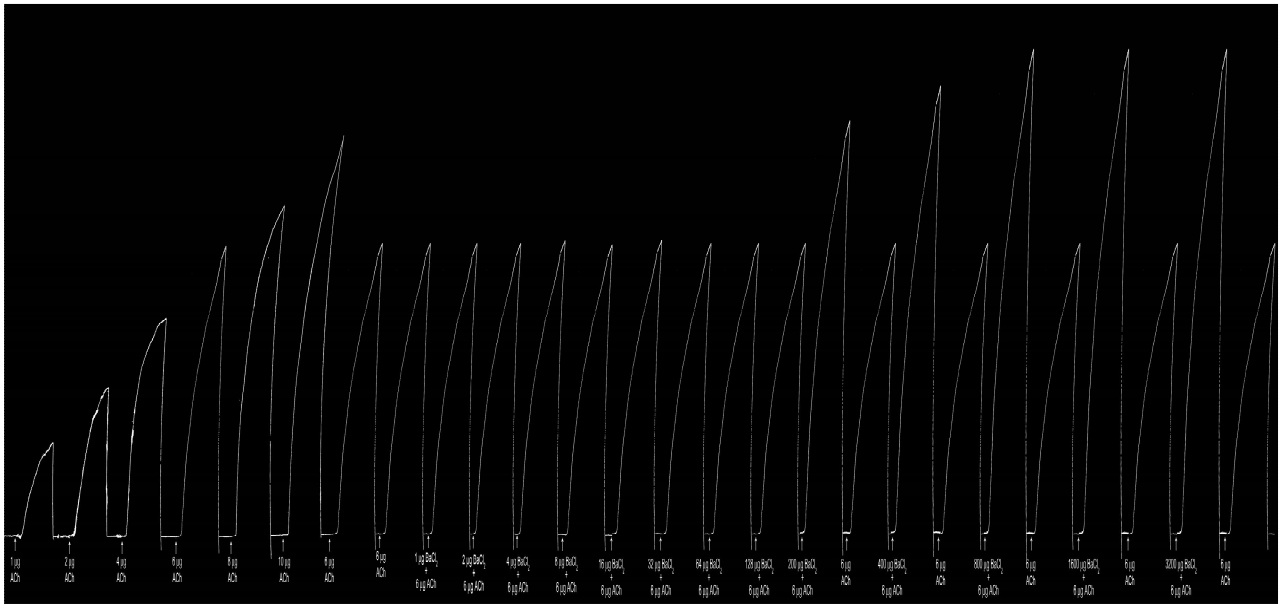

Figure.2. Effect of $BaCl_2$ on skeletal muscle contraction produced by acetylcholine in frog

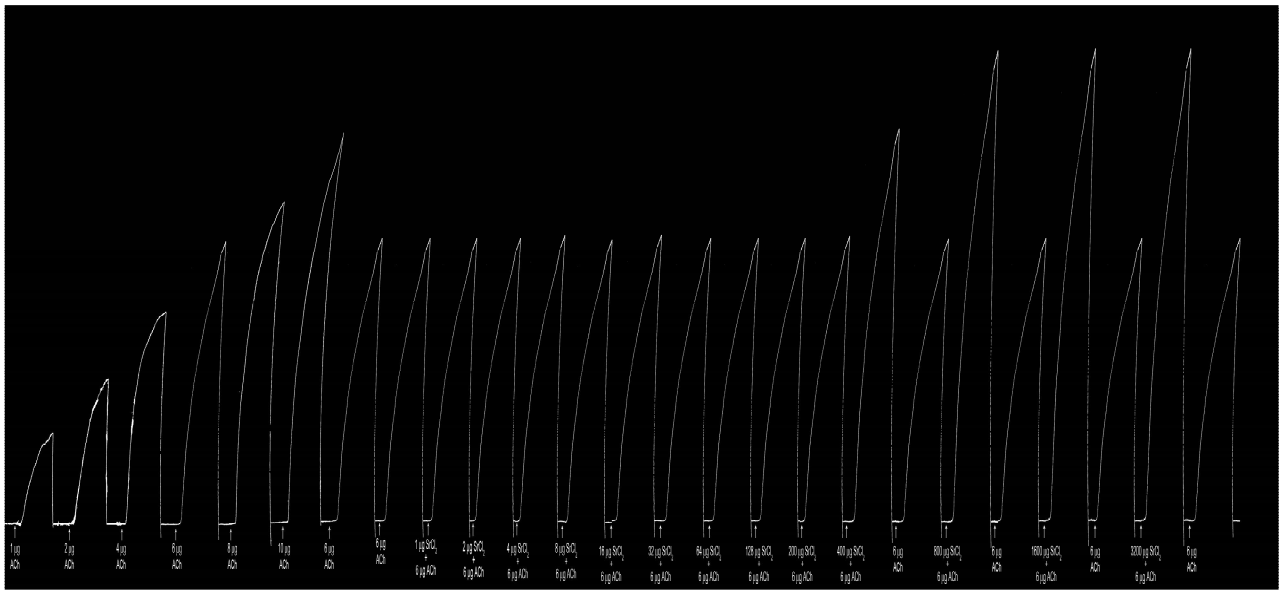

Figure.3. Effect of $SrCl_2$ on skeletal muscle contraction produced by acetylcholine in frog

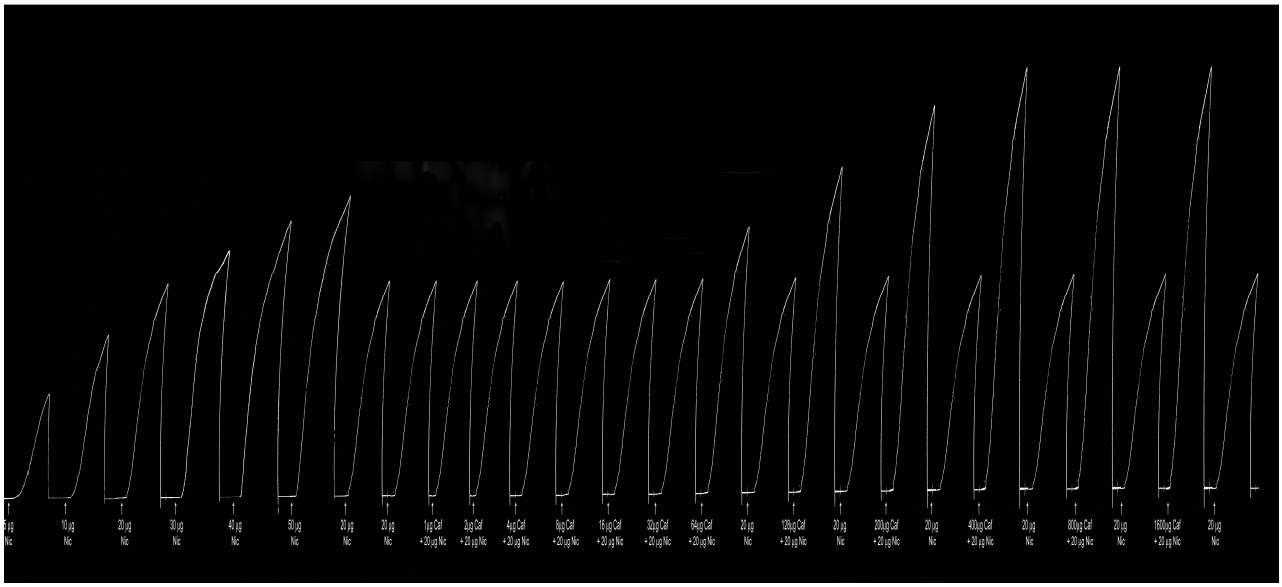

Figure.4. Effect of $CaCl_2$ on skeletal muscle contraction produced by nicotine in frog

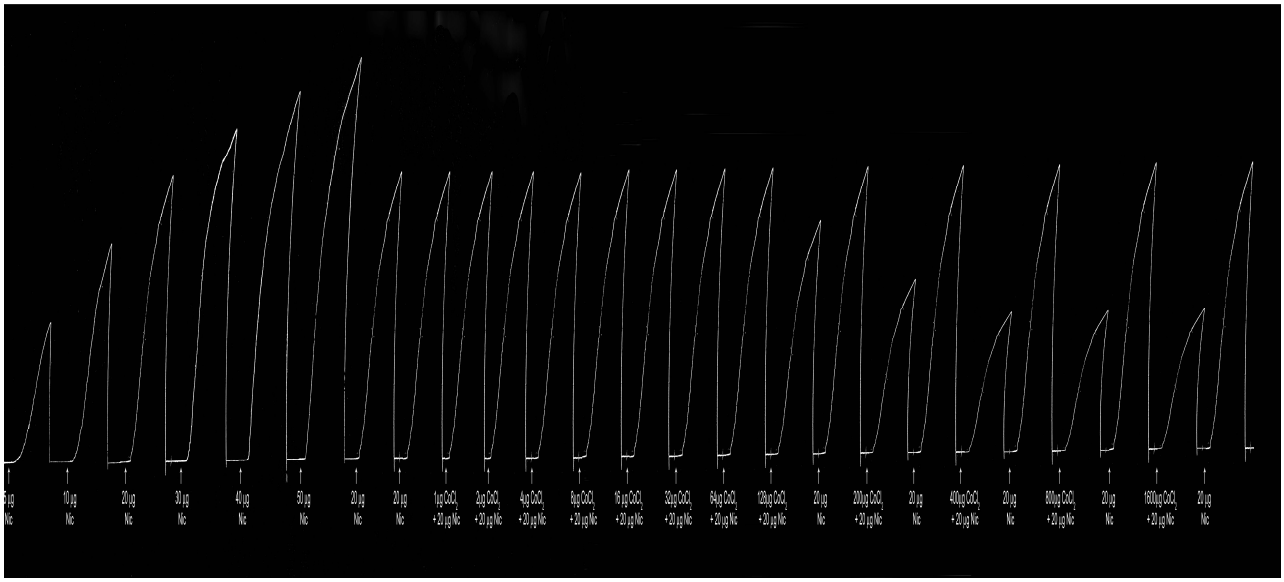

Figure.5. Effect of $CoCl_2$ on skeletal muscle contraction produced by nicotine in Frog

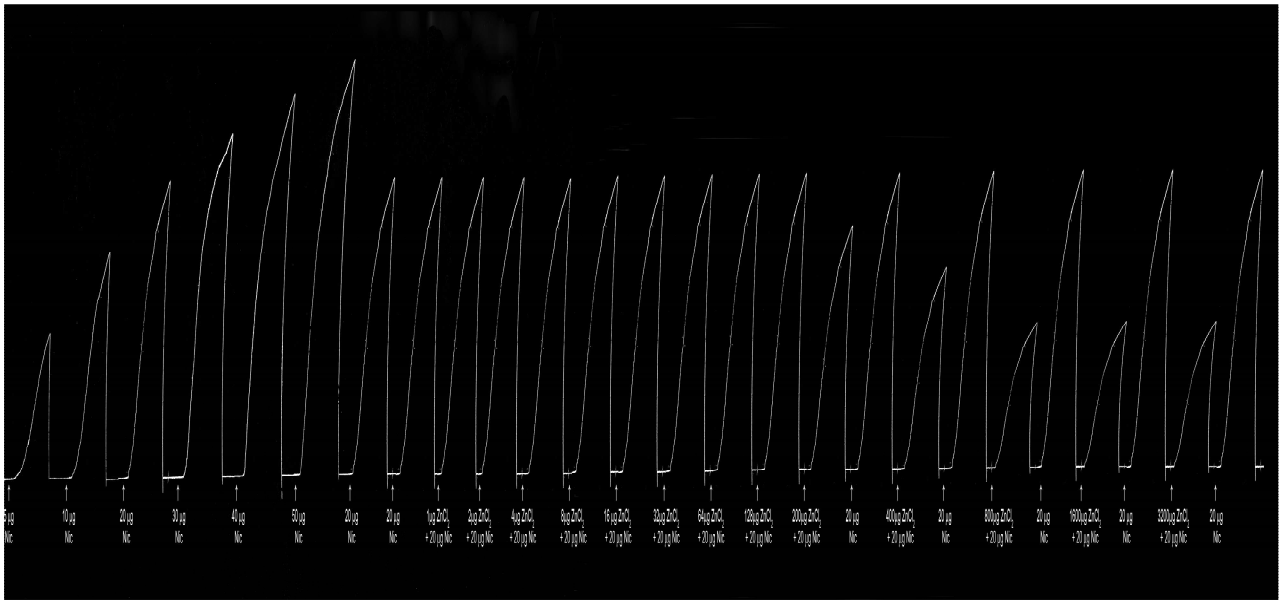

Figure.6. Effect of $ZnCl_2$ on skeletal muscle contraction produced by nicotine in frog