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Caffeine Alters Skeletal Muscle Contraction by Opening of Calcium Ion Channels

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Abstract: The aim of this study was to investigate the effect of caffeine on the amplitude and rate of skeletal muscle contraction using frog sciatic nerve-gastrocnemius muscle model. Caffeine is a xanthine alkaloid whose use is widely unregulated. It is taken as a central nervous system stimulant in various foods and drinks. The effect of caffeine on skeletal muscle contraction and a possible elucidation of its mechanism of action were investigated. The sciatic nerve-gastrocnemius muscle preparation of the frog mounted on a kymograph was utilized. Varying doses of caffeine was added to the organ bath at 5, 10, 15, 20 and 25 mg/mL and its effect on skeletal muscle contraction was studied. The effects of caffeine preceded by administration of acetylcholine, atropine, nifedipine, magnesium chloride and calcium gluconate at 25 mg/mL were also studied. A dose dependent increase in skeletal muscle contraction (25.25±0.48, 49.00 ±1.23, 52.38±2.58, 59.25±1.11 and 68.50±0.87 mV; $p<0.05$) was observed on administration of increasing doses (5, 10, 15, 20 and 25 mg/mL, respectively) of caffeine respectively. While a significant reduction (0.90±0.04 mV) and increase (77.50±1.56 mV) in strength of contraction was observed on administration of nifedipine and calcium gluconate respectively. Administration of magnesium chloride caused a significant decrease in the strength of contraction (28.25±5.01) as compared to control. However, there was no significant difference in the contraction period and relaxation period between the treatment groups. The findings imply that caffeine increases skeletal muscle contraction and suggests it exerts the effect through increasing calcium ion release.

Key words: Caffeine, calcium ion, magnesium chloride, nifedipine, skeletal muscle

INTRODUCTION

Caffeine is a bitter crystalline xanthine alkaloid found in varying quantities in beans, leaves and fruit of over 60 plants where it acts as a natural pesticide. It is consumed by humans in infusions extracted from the beans of the coffee plant and the leaves of the tea bush to form various foods and drinks derived from kolanut or from cocoa (Peters, 1967; Richard, 2007).

In humans, caffeine is a Central Nervous System (CNS) stimulant having the effect of temporarily warding off drowsiness and restoring alertness. Beverages containing caffeine and "energy" drinks enjoy great popularity among long distance drivers and students preparing for examinations (Rubbin and Rita 2008; Kennedy *et al*., 2007; Antonio *et al*., 1999).

Kolanuts, a major source of caffeine is consumed widely in Northern Nigeria and there is a steady influx of "energy" drinks into the Nigeria market recently. One of such drink is "Red Bull" which contains about 84mg/ml of caffeine. Caffeine is the world's most widely consumed psychoactive substance but it is legal and unregulated in nearly all jurisdictions (Han *et al*., 2007; Schmidt, 2005; Kerrigan, 2005; Juliano, 2004; Mrvos, 2004; Horgan, 1990; Trice and Haymes, 1995; Kamijo *et al*., 1999; Holtzman *et al*., 1991; Green and Stiles, 1986; James and Stirling, 1983).

The CNS stimulating effects are well documented, such as anxiety and sleep disorders (James and Stirling, 1983), memory and learning disorders (Han *et al*., 2007; Green and Stiles, 1986), tolerance and withdrawal (Horgan, 1990; Lesk and Womble, 2004). Its cardiovascular effects are also reported (Schnoll, 2007; Greenberge *et al*., 2007). There is a paucity of literature on the effect and mechanism of action of caffeine in neuromuscular transmission and skeletal muscle contraction.

The study was aimed at studying the effect of caffeine (varying doses of 5-25g/mL) on skeletal muscle contraction and the possible mechanism of action using the isolated Frog Sciatic Nerve-Gastrocnemius Muscle preparation.

METHODOLOGY

This study was conducted during the month of April 2011 in the laboratory of Human Physiology Department, Faculty of Medicine in Ahmadu Bello University, Zaria, Nigeria.

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Table 1: Effect of caffeine on frog skeletal muscle contraction

	Latent period (s)	Contraction period (s)	Relaxation period (s)	Strength of contraction (mV)
Control (Ringer solution)	0.03±0.00	0.11±0.01	0.08±0.02	44.20±7.44
Caffeine 5 mg/mL	0.03±0.00	0.09±0.00	0.08±0.00	25.25±0.48
Caffeine 10 mg/mL	0.02±0.00	0.12±0.00	0.09±0.00	49.00±1.23
Caffeine 15 mg/mL	0.02±0.00	0.13±0.00	0.09±0.00	52.38±2.58*
Caffeine 20 mg/mL	0.02±0.00	0.15±0.00*	0.09±0.00	59.25±1.11*
Caffeine 25 mg/mL	0.02±0.00	0.15±0.00*	0.09±0.03	68.50±0.87*

*: p>0.05; p<0.001; p>0.05; p<0.001

Table 2:Effect of caffeine, acetylcholine, atropine, calcium and magnesium on muscle contraction

Treatment	Latent period (s)	Contraction period (s)	Relaxation period (s)	Strength of contraction (mV)
Control (Ringer's solution)	0.03±0.01	0.11±0.09	0.08±0.02	44.20±7.44
Caffeine (20 mg/mL)	0.02±0.00	0.15±0.00	0.09±0.00	59.25±1.11*
Ach (25 mg/mL)+ Caffeine (20 mg/ml)	0.02±0.00	0.15±0.02	0.09±0.00	53.50±3.04
Calcium Gluconate + Caffeine (20 mg/mL)	0.02±0.00	0.17±0.01	0.09±0.00	77.50±1.56*
Atropine + Caffeine (20 mg/mL)	0.02±0.00	0.15±0.00	0.29±0.19	36.25±2.72
$MgCl_2$ (25 mg/mL) + Caffeine. (20 mg/mL).	0.02±0.00	0.08±0.02	0.08±0.01	28.25±5.01**
Nifedipine 25 mg/mL + Caffeine (20 mg/mL)	0.06±0.00*	0.09±0.00	0.06±0.00	0.90±0.04**
	*p<0.001	p>0.05	p>0.05	**p<0.001

The Sciatic nerve-Gastrocnemius muscle of a frog was dissected, mounted on a Student Kymograph and bathed in 50 mL of frog Ringer solution. Simple muscle contractions were recorded on the Kymograph papers as control. The strength of contraction, duration of contraction and latent period were recorded.

The effect of caffeine (Batch No. 63774170, BDH, England) at doses of 5, 10, 15, 20, 25 mg/mL were studied on the muscle contraction (5 contractions for each dose were recorded) Table 1 and Fig. 1, 2.

The effect of technical grade of acetylcholine, atropine, nifedipine, magnesium chloride and calcium gluconate at 25 mg/mL each was studied by adding 1ml of each alone and recording 5 contractions and 1mL of each before 1ml of 25mg/mL of caffeine and recording 5 contractions.

The ringer solution on the nerve bath was changed and the tissue preparation "washed" twice before addition of the next standard drug alone and followed by caffeine. From each contraction, the latent period, contraction period, relaxation period (s) and strength of contraction (mV) were recorded.

Statistical analysis: Results were recorded as mean±SEM and analyzed using ANOVA. $p<0.05$ were considered statistically significant.

RESULTS

Results show a statistically significant dose dependent increase ($p<0.001$) in the contraction period and strength of contraction from 15 to 25 mg/mL. The latent period and relaxation period were not affected by caffeine. (Table 1, 2 and Fig. 1, 2).

When nifedipine was added to the nerve bath before addition of caffeine, the Latent period of 0.06±0.00s was significantly prolonged ($p<0.001$) when compared with the control and with caffeine (20 mg/mL) respectively (Table 2 and Fig. 1 and 6).

Similarly addition of Nifedipine before caffeine to the nerve bath caused a statistically significant redaction of the strength of contraction from 44.20±7.44 MV in control and 29.25±1.11MV in the caffeine groups (20 mg/mL) to 0.90±0.04MV ($p<0.001$), (Fig. 6). Addition of calcium glucomate (25 mg/mL) before caffeine caused a statistically significant rise ($p<0.001$) in the strength of contraction to 53.50±3.04MV, (Table 2 and Fig. 5). Magnesium chloride caused a significant decrease in the strength of contraction to 28.25±5.01MV $p<0.001$, (Fig. 4B). There was no significant difference in the contraction period and relaxation period between the treatment groups (Table 2 and Fig. 2). Pretreatment with Acetylcholine and Atropine before Caffeine did not significantly alter the latent period, amplitude of contraction and duration of contraction and relaxation ($p>0.05$), (Fig. 3 and 4A).

DISCUSSION

Caffeine is widely consumed in foods and drinks and though it is a psychoactive stimulant its use is largely unregulated. The effects of long time use of caffeine on the Central Nervous System (CNS) include anxiety, sleep disorders, memory lethargy, tolerance and withdrawal syndromes including features of acute intoxication (´caffeine jitters´) are well reported.

In the present study caffeine caused a dose dependent significant increase in force of contraction and duration of contraction ($p<0.001$) of skeletal muscle. There was no

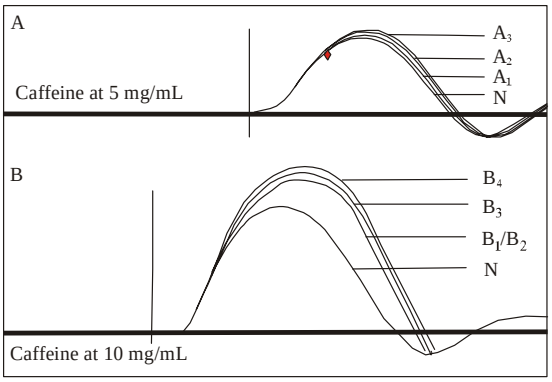

Fig. 1: Effect of caffeine at 5 mg/mL (A) and at 10 mg/mL (B) on frog gastronecmius muscle contraction

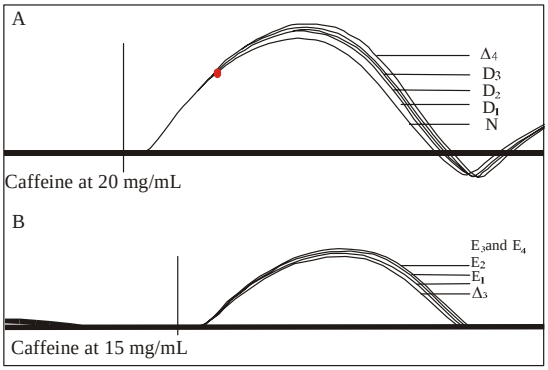

Fig. 2: Effect of caffeine at 20 (A) and 15 mg/mL (B) on frog gatrocnemius musle contraction

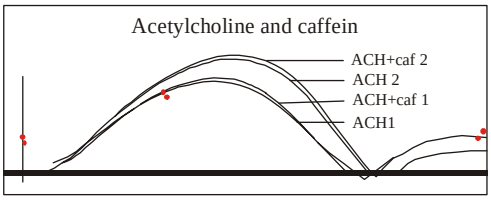

Fig. 3: Effect of pretreatment with acetylcholine followed by caffeine on frog skeletal muscle contraction

significant effect on the latent period and relaxation period. This effect is potentiated with calcium gluconate. Nifedipine, a calcium ion channel blocker caused a significant prolongation of the latent period and reduction in the force of contraction ($p<0.001$) suggesting that caffeine acts through the release of calcium ion from the terminal cisterns of the sarcoplasmic reticulum. More calcium binds to troponin C leading to uncovering of binding sites on actin filament for myosin, causing a power stroke. Increased concentration of calcium ion will produce a "Rachet theory"- an increased number of power

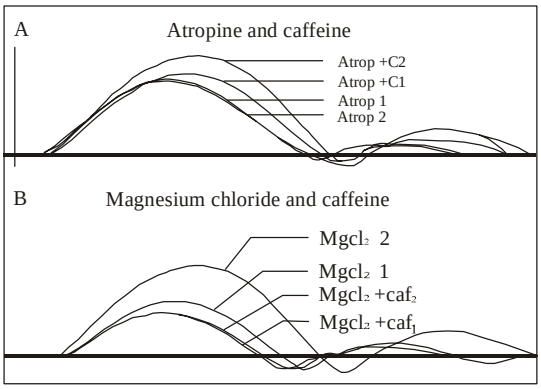

Fig. 4 A, B: Effect of pretreatment with atropine (A) and magnesium chloride (B) followed by caffeine on frog skeletal muscle contraction

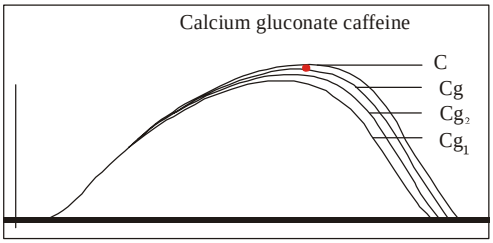

Fig. 5: Effect of pretreatment with calcium gluconate on frog skeletal muscle contraction

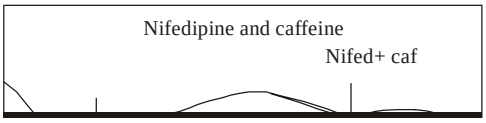

Fig. 6: Effect of Pretreatment with Nifedipine on caffeine induced skeletal muscle contraction in Frog

strokes and force of contraction - and increase the number of crossbridges. Magnesium chloride, a calcium ion channel blocker which acts by competing with calcium ion on binding sites also caused a significant decrease in force of contraction ($p<0.05$). The effect of caffeine on skeletal muscle contraction is not through nicotinic or muscarinic receptor because pretreatment with acetylcholine did not cause any significant effect on the latent period, strength of contraction, contraction power or relaxation period and atropine an acetylcholine receptor blocker also did not affect the muscle contraction. Thus caffeine did not exert its effect on skeletal muscle contraction on the calcium ion at the neuromuscular junction and it is not prone to destruction by acetylcholinesterase.

The action of caffeine on the calcium ion release in skeletal muscle may be responsible for peripheral manifestation of caffenism such as tremulousness, muscle twitching and hyperreflexia and may be managed with

ryanodine receptor antagonist Dantrolene, Ruthenium, Procaine and Tetracaine (Xu *et al*., 1998; Vites and Pappano, 1994).

Physical coupling to voltage sensitive dihydropyridine receptor (RyR1) transmits the voltage-mediated signal to ryanodine receptors (RyRs) which is the major cellular mediator of calcium induced calcium release in cells (Zuchi and Ronca-Testoni, 1997).

It has been shown that calcium release from a number of ryanodine receptors in a ryanodine receptor cluster results in a spatio-temporally restricted rise in cytosolic calcium that can be visualized as a "calcium spark" (Cheng *et al*., 1993). Ryanodine receptors are similar to inositol triphosphate (IP_3) receptor, and stimulated to transport Ca^{2+} into cytosol by recognizing Ca^{2+} on its cytosolic side, thus establishing a positive feedback" mechanism, a small amount of Ca^{2+} in the cytosol near the receptor will cause it to release even more Ca^{2+} (calcium-induced calcium release/CICR). The localized and time - limited activity of Ca^{2+} in the cytosol is also called a "Ca^{2+}-wave". The building of the wave is through the feedback mechanism of the ryanodine receptor and the activation of phospholipase C by GPCR or TRK which leads to production of inositol triphosphate, which in turn activates the IP_3 receptor.

It is concluded that caffeine causes a dose dependent increase in force of contraction and duration of contraction of skeletal muscle by causing increase release of calcium ions from the sarcoplasmic reticulum through a physical coupling and conformational changes in the voltage sensitive dihydropyridine receptor. This action of caffeine was blocked by calcium ion channel blocker nifedipine and magnesium chloride. Caffenism can be managed by modifying the activities of Ryanodine receptors by using its antagonists or agonists.

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