EFFECT OF CAFFEINE ON FROG MUSCLE

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ABSTRACT

Aim: *The aim of the present investigation is to study the effects of caffeine on frog skeletal, cardiac and smooth muscle tissues.*
Material and methods: *In this study muscle strips were obtained from Sartorius muscle, heart muscle and rectum of frog after pithing the animal. Caffeine (30mM) was administered in aqueous form with the help of Micropipette directly upon the isolated muscle sample. These isolated samples were prepared, fixed and analyzed with the help of laser beam where linear movement of muscle contraction is converted to angular movement which was recorded on the graph paper and further statistical analysis was done using student's t-test to determine significance of differences between means. A "p" value of < 0.05 was considered significant.*
Conclusion: *Caffeine (30mM) induces sustained contraction in skeletal and smooth muscle, but cardiac muscle does not respond to caffeine though it increases the natural beat of sinoatrial preparation.*

Keywords: *Caffeine, laser beam, student's t-test, pithing*

INTRODUCTION

Caffeine is the most widely consumed psychoactive 'drug' in the world and probably one of the most commonly used stimulants in sports. Caffeine is an alkaloid obtained from tea, coffee and cola drinks, which contains variable amounts of caffeine or related substances with similar effects as that of caffeine. The main effect of caffeine is to enhance the muscular performance, so called "Ergogenic effect". Caffeine is known to produce contractions in skeletal muscles.[1]

While the application of Caffeine at concentrations higher than 5mM to the skeletal muscle produces a strong contraction,[2] lower concentrations of caffeine do not cause an appreciable contraction.[3] The main target of this compound seems to be the sarcoplasmic reticulum (SR) from which release of calcium occurs leading to contraction of the muscle tissue. The stimulus for SR calcium release is depolarization of the transverse tubules (TT). It has been proposed that this coupling may involve a direct molecular link between the TT voltage sensors and the SR channels.[4]

Caffeine seems to promote this release of calcium thereby enhancing the contractility of skeletal muscle in a variety of species and a few studies have reported its effect on cardiac as well as smooth muscle.[1, 5] In the present study we have investigated the effect of caffeine on frog skeletal, cardiac and smooth muscle tissues.

MATERIAL AND METHODS

Frogs belonging to the species *Rana tigrina* of both sexes, weighing between 75-120 g were used for the study. There were four groups each with a sample size of six. One group was for skeletal muscle, one for smooth muscle and two for cardiac muscle tissue.

The frogs were anaesthetized with ether prior to pithing and preparations were taken from Sartorius muscle (10mm x 5 mm), from the heart (circular rings were made from the lower end of the ventricle and also from the atria close to the white crescentic line) and from the rectum (10 mm x 5 mm). The muscle strips were isolated and immediately transferred in a Petri-dish containing Frog Ringer solution.[5] The composition of Frog Ringer in mM L^{-1} is as follows.

Sodium Chloride (Na Cl)-117, Potassium Chloride (K Cl)-3, Calcium Chloride (Ca Cl)-1, Magnesium Chloride (Mg Cl2)-1, Monosodium Phosphate (NaH_2PO_4)-0.2, Disodium Phosphate (Na_2HPO_4)-0.8, 10 Glucose, pH adjusted to 7.4 with Sodium Hydroxide(NaOH).[6, 7]

The muscle strips, after being washed thoroughly, were placed in the specially designed recording device and further recordings were made.

Recording devices:

An inexpensive, sensitive laser based isotonic recording system has been designed to observe and measure muscle contraction.[8] In this method the linear movement of muscle contraction is converted to angular movement of a lever that could be measured accurately with laser light diffraction by using graph paper.[9] The light is diffracted once by an optical device and then this diffracted linear movement of light is recorded on the graph paper. The laser light which is focused on the mirror moves

and the refracted image of this movement is magnified and recorded on the graph paper. The recording device is designed in such a manner that on a fiber plate of size 7.5 cm x 3.5 cm, there stands a central bath for muscle perfusion, where the muscle strip is attached to two tiny metallic clips, one of which is fixed and the other clip is further attached with flexible spring.

Another device is designed in such a manner that its tail part is resting on the spring and having a circular movement whenever the spring is stretched. In the centre of this device a tiny mirror is fixed where laser light is focused to this mirror and its reflected image is recorded on a graph paper. The laser source and the recording graph paper subtend an equal angle with the mirror and also are equidistant from the mirror.

One end of the muscle was attached to a fixed clip, which cannot move and the other end of the muscle which is attached through the spring can move and stretch the spring whenever it contracts and the muscle length shortens. The linear movement of the spring is converted to a rotatory movement of the mirror device which is attached to it. The laser light which is focused on the mirror moves and the reflected image of this movement is magnified and recorded on the graph paper. The magnification is proportional to the distance of the graph paper from the mirror. The distance was so adjusted that for 1mm muscle shortening, the laser spot on the graph paper moved by 35 mm, hence the magnification factor was 35. This calibration was done before every recording was made.

At first the muscle preparation were mounted in the bath and perfused with the same frog ringer solution as mentioned above using a small syringe. The tissues were allowed to settle for 5 minutes and any shortening during this period was noted. After the baseline recording, changes occurring in the muscle tissue were observed after administration of the caffeine. Aqueous Caffeine solution of 30 mM concentration was directly added in the form of drops using micropipette over the isolated perfused muscle preparation. The concentration of caffeine that was used is **30 mM.**

The parameters that were recorded are

1. Change in length or shortening (in mm)
2. The change in rate of contractions, in case of rhythmic contractions seen in sinoatrial preparations.
3. The time taken for attaining maximum response (in seconds)

The parameters were analyzed under control conditions and test conditions and compared. The comparison was also made across different types of muscle tissues.

Institutional Animal Ethical Committee (IAEC) guidelines for the care and use of laboratory animals were followed.

Sample recording: After preparing the tissue all the instruments were arranged as shown in the fig.1 Caffeine (30mM) was administered in the form of drops using micropipette over the isolated perfused muscle preparation.

The muscle preparation started showing response after a latent period of 2 seconds and maximum response was seen after 7 seconds. The total recording time was of 25 seconds. The consecutive recordings were done after a rest period of 5 minutes and every recording was done for 25 seconds each.

Fig. 1: Muscle activity recording using laser beam

STATISTICAL ANALYSIS

The data recorded is expressed as mean ± SD and compared with unpaired student's t-test to determine significance of differences between means. A "p" value of < 0.05 was considered significant.

RESULTS

After complete relaxation of muscles for five minutes, aqueous form of Caffeine was directly administered over the muscle preparation with the help of micropipette which produced contractions in the form of shortening of the muscles. These contractions were recorded with the help of laser light upon a graph paper. The response started after a latent period of less than 2 seconds and the maximum effect was seen a few seconds later. The time taken for starting of contractions and the time taken for reaching the peak of contraction (maximum shortening) has been shown in table 1.

Table no. 1: Effect of caffeine on skeletal muscle

Number	Time for onset (sec)	Time for peak (sec)	Shortening (mm)
1	2	7	1.428
2	1	11	1.857
3	2	8	1.142
4	2	8	1.500
5	2	3	0.571
6	1	10	1.000
Mean	1.666667	7.833333	1.249667
SD	0.516398	2.786874	0.44673

The response of the smooth muscle to addition of caffeine was similar but with a significant delay in the time taken for attainment of the peak response (7.8±2.8 versus 15.1±2.1, p=0.0005). The amount of shortening produced in the smooth muscle was also significantly less than that seen in skeletal muscle (1.2±0.4 versus 0.4±0.1, p=0.005). These are shown in table 2 and figures 1 and 2.

Table no. 2: Effect of caffeine on Smooth muscle

Number	Time for onset (sec)	Time for peak (sec)	Shortening (mm)
1	2	13	0.571
2	2	16	0.500
3	2	17	0.357
4	2	16	0.285
5	2	12	0.500
6	2	16	0.357
Mean	2	15.16667	0.428333
SD	0	2.136976	0.110768

The ventricular muscle of the frog failed to respond to caffeine, but the sinus venosus with a small strip of atrial muscle which was showing spontaneous beat showed a change in the rate of beats in response to addition of caffeine. The rate which was slow initially, increased significantly after the addition of caffeine (34.2±2.6 beats versus 48.5±2.3 beats, p=0.000005) and the amplitude was also increased though it was not quantified. These results are shown in table 3.

Table no. 3: Effect of caffeine on cardiac muscle of beating frog heart

Number	Time for onset (sec)	Rate before caffeine (Beats/ min)	Rate after caffeine (Beats/ min)
1	12	32	45
2	14	36	49
3	11	32	48
4	14	38	52
5	13	32	49
6	12	35	48
Mean	12.66667	34.16667	48.5
SD	1.21106	2.562551	2.258318

DISCUSSION

The method of recording the muscle contractions employed in the present study by using the laser beam is fairly accurate and is comparable to computer based edge detection systems which are highly expensive. This method is not only user friendly and simple but is extremely cost effective. It is highly sensitive and is able to record minute changes in the muscle length. Thin short bundles of muscle can be used, and can be perfused ensuring quick access to drugs or ions present in the perfusate.

Our results confirm the original finding that caffeine induces a sustained contraction (contracture) in the skeletal muscle. Similar effect was noticed in the isolated smooth muscle preparation namely the frog rectum which is commonly used in many laboratories as a typical smooth muscle preparation for demonstration of actions of various bioactive agents. However our results indicate that the effect of caffeine (30mM) on frog skeletal muscle is faster and the extent of muscle shortening is more in comparison to the effect of caffeine on smooth muscle. While the exact reason for this difference is not clear, it is generally believed that the less abundance of sarcoplasmic reticulum in the smooth muscle from which calcium is released by caffeine may be responsible for this. In addition the enzymatic steps involved downstream may also be slow in the smooth muscle.

In this study it was found that ventricular muscle strip preparation of the amphibian heart failed to respond to caffeine, but the beating sinoatrial muscle strip when treated with caffeine (30mM) showed an increase in frequency as well as in amplitude of contractions. These effects are difficult to explain. Histological and biochemical studies has shown that sarcoplasmic reticulum is absent in the amphibian ventricle.[10] Thus the finding of this study can be interpreted on the basis of the above report of the lack of sarcoplasmic reticulum in the frog ventricular muscle.

Increase in the rate of normal beat of the sinoatrial preparation by caffeine treatment was also observed in the present study. This indicates that the musculature of the sinus venosus and the atria of the frog are different from the frog ventricular muscle in their sensitivity to caffeine. It is speculated that unlike the ventricular muscle, caffeine sensitive receptors are present in sinoatrial preparation. Further experimentation is required to prove this hypothesis. Our study here clearly shows that, caffeine increases the contraction ability of skeletal muscles; this is why caffeine comes under the list of performance enhancing drugs. This also supports the fact that why professional athletes are prohibited from using excessive amount of caffeine.

CONCLUSION

1. The recording system is simple and adequate to monitor the length changes in small muscle strips of skeletal, cardiac and smooth muscles of the frog.
2. Caffeine induces a sustained contraction as reported in the previous studies in the frog skeletal muscle.
3. Effect of caffeine on frog rectum is similar in action but the time course is much slower.
4. The ventricular muscle of frog did not respond to caffeine.
5. Caffeine potentiated the natural beat of sinoatrial preparation of the frog.

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