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EFFECT OF AMINOPHYLLINE ON MUSCLE FATIGUE

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Abstract: The effects of two different concentrations of aminophylline, 50 μM/L and 500 μM/L on muscle fatiguability were tested using frog gastrocnemius–sciatic preparations. Two stimulation protocols, one high energy demand, and the other low energy demand were used to induce muscle fatigue. The indices of fatigue employed were (a) the decrease in peak tetanic contraction, (b) the increase in half–contraction time and (c) the increase in the contraction period in response to a high–energy–demand stimulation protocol of fatigue–induction. At the same time, it prolongs the increase in relaxation$_{50-30}$ time in response to the same protocol.

Key words : frog muscle, drug effect, gastrocnemius–preparation, fatigue–resistance

INTRODUCTION

Aminophylline, a methylxanthine is a bronchodilator and central nervous stimulant and also produces direct effects on skeletal and smooth muscle (1, 2). Aminophylline is known to increase twitch and submaximum tetanic force generation (1, 2, 3) and increase the maximum velocity of shortening (4, 5). The effect of aminophylline on the velocity and power of muscle shortening makes some concomitant variability in muscle fatiguability a likely possibility. A study was therefore carried out to investigate the effect of aminophylline on muscle fatiguability.

METHODS

The study was carried out on 15 frog gastrocnemius–sciatic preparations. The isometric muscle contractions were recorded on a Single–Channel Physiograph (INCO) using a tension transducer. The response of muscles to fatigue resulting from repeated contractions were tested using three patterns of maximal stimulation (6), delivered using a stimulator (NIHON KOHDEN) (SEN 3201) connected with a Timer (INCO).

(a) Test stimulation: It consisted of a train of 400 stimulations at 100 Hz given over 4s.

(b) Protocol–1: It consisted of 30 maximal tetanic contractions, each of 4s duration, elicited every 12s (duty cycle 1:3). A stimulation frequency of 100 Hz was used.

(c) Protocol–2: It consisted of 180 brief tetanic contractions, 0.25s long and elicited every 5s (duty cycle 1:20). stimulation frequency was 30 Hz, which gave fused contractions but produced only 60% maximum force.

The parameters used to assess muscle fatigue were:

(a) The peak tetanic contraction (PT): The peak contractions were measured during the "test" contractions elicited at the beginning, and after each of the two stimulation protocols.

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(b) The half–contraction time (CT): This was taken as the time after start of stimulation for force to reach 50% maximum force during a test contraction.

(c) The rate of relaxation$_{50-30}$ (RT): This was taken as the time taken for the force of contration to decline from 50% to 30% of the peak valued during the test contractions.

(d) Fatigue resistance (ratio of the peak tetanic contraction before and after the stimulation protocol).

The experiment was conducted in two groups: the control (Group–A) and the test groups (B & C). In the test group, Aminophylline was added to the Ringer solution bathing the nerve–muscle preparation. Two strengths of Aminophylline was used (a) 50 µM/L (Group–A) and (b) 500 µM/L (Group–B). Fatiguability was tested after allowing a 30–minute equilibrium period (7).

RESULTS

The peak contraction, half–contraction time and relaxation$_{50-30}$ time for the different groups at rest and after fatiguing stimulation are given in Tables Ia and Ib. Aminophylline did not appear to have any effect on the peak contraction, half–contraction time and relaxation$_{50-30}$ time of the resting muscle as analysed by Wilcoxon Matched Pair Signed Rank Test. However, aminopyhlline did appear to affect some if not all of the post–fatigue changes. The Kruskal–Wallis' Test and Mann–Whitney U Test showed that the increase in the half–contraction and relaxation$_{50-30}$ periods following muscle fatigue were susceptible to the effects of aminophylline (Table II). In general, the results of our study indicate that aminophylline in high concentration (500 µM/L) minimises the increase in half–contraction period of isolated frog gastrocnemius muscle preparations in response to a high–energy–demand stimulation protocol of fatigue–induction. At the same time, it prolongs the increase in relaxation$_{50-30}$ time in response to the same protocol. Aminophylline did not affect the fatigue–induced changes in the peak tetanic contraction, the half contraction time or the relaxation$_{50-30}$ time.

TABLE I (a): The mean ±SD values of peak contraction, half–contraction time and relaxation$_{50-30}$ time in Groups A, B and C, measured before a fatiguing stimulation, and after fatiguing stimulation protocol 1.

	Parameter	*Group–A*	*Group–B*	*Group–C*
1	Peak contraction in control		0.57 ± 0.13	0.80 ± 0.16
2	Half–contraction time in control		0.05 ± 0.02	0.05 ± 0.01
3.	Relaxation$_{50-30}$ time in control		0.07 ± 0.04	0.05 ± 0.02
4.	Peak contraction before protocol 1	0.58 ± 0.04	0.62 ± 0.12	0.81 ± 0.16
5.	Half–contraction time before portocol 1	0.04 ± 0.01	0.05 ± 0.02	0.05 ± 0.01
6.	Relaxation$_{50-30}$ time before protocol 1	0.06 ± 0.01	0.06 ± 0.05	0.05 ± 0.02
7.	Fatigue resistance in protocol 1	48.30 ± 19.40	48.40 ± 16.20	0.60 ± 4.09
8.	Peak contraction after protocol 1	0.25 ± 0.08	0.34 ± 0.20	0.33 ± 0.16
9.	Half–contraction time after protocol 1	0.12 ± 0.03	0.12 ± 0.03	0.08 ± 0.01
10.	Relaxation$_{50-30}$ time after protocol 1	0.06 ± 0.01	0.07 ± 0.04	0.12 ± 0.01

TABLE I (b): The mean ±SD values of peak contraction, half-contraction time and relaxation$_{50-30}$ time in Groups A, B and C, measured before a fatiguing stimulation, and after fatiguing stimulation protocol 2.

	Parameter	*Group-A*	*Group-B*	*Group-C*
1	Peak contraction in control		0.57 ± 0.13	0.80 ± 0.16
2	Half-contraction time in control		0.05 ± 0.02	0.05 ± 0.01
3.	Relaxation$_{50-30}$ time in control		0.07 ± 0.04	0.05 ± 0.02
4.	Peak contraction before protocol 2	0.50 ± 0.03	0.57 ± 0.07	0.73 ± 0.16
5.	Half-contraction time before portocol 2	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.02
6.	Relaxation$_{50-30}$ time before protocol 2	0.07 ± 0.01	0.11 ± 0.03	0.11 ± 0.01
7.	Fatigue resistance in protocol 2	10.40 ± 1.50	8.80 ± 4.10	7.96 ± 1.58
8.	Peak contraction after protocol 2	0.33 ± 0.05	0.34 ± 0.09	0.45 ± 0.22
9.	Half-contraction time after protocol 2	0.10 ± 0.01	0.12 ± 0.04	0.14 ± 0.02
10.	Relaxation$_{50-30}$ time after protocol 2	0.09 ± 0.01	0.16 ± 0.02	0.13 ± 0.01

TABLE II : Comparision of fatigue-induced changes (i.e. changes in peak contraction, half-contraction time and relaxation$_{50-30}$ time) in Group A, B and C by the Kruskal-Wallis' Test. Significance of multiple comparison is by Mann-Whitney 'U' Test where comparison is significant.

Indices of Muscle Fatigue	*Inter-group comparisons Significance at P = 0.05*
Fatigue resistance in protocol 1	Not significant
Fatigue resistance in protocol 2	Not significant
Change in peak contraction after protocol 1	Not significant
Change in half-contraction time after protocol 1	Group A & C are significantly different (P<0.001)
	Group A & B are not significantly different
Change in relaxation$_{50-30}$ time after protocol 1	Group A & C are not significantly different (P<0.001)
	Group A & C are significantly different
Change in Half-contraction time after protocol	Not significant
Change in relaxation$_{50-30}$ time after protocol 2	Not significant
Change in relaxation$_{50-30}$ time after protocol 2	Not significant

DISCUSSION

Aminophylline is known to increase the twitch and submaximal tetanic force generation of mammalian diaphragm (1, 2) and frog semitendinosus muscle (3). Aminophylline is known not to affect maximum tetanic force of mammalian (2) or frog muscle (3).

In our study, we have used two protocols of fatigue stimulation, but although both of them were tetanic stimulations, both caused submaximal contractions (The stimulation frequencies were 100Hz and 30Hz).

Some of the effects of aminophylline can be inhibited by removing extra-cellular calcium or

by blocking the influx of calcium through the plasma membranes (1, 2). Several groups have proposed that aminophylline increases the force at submaximal stimulation by increasing calcium influx across the sarcolemma during excitation–contraction coupling (1, 2, 8, 9). In addition, at submaximum frequencies of stimulation, increased calcium influx may increase calcium release from the sarcoplasmic reticulum by a calcium–dependent calcium release mechanism (9).

The potentiation of submaximum force development by aminophylline has been shown to be inhibited by calcium channel blockers (1). Consequently, aminophylline appears to enhance the force development of submaximally activated muscle by increasing intracellular calcium (11) and activation additional crossbridges (2, 3).

It is possible that aminophylline minimises the fatigue–induced increase in contraction time by increasing the entry of Ca^{++} into the sarcoplasm. The prolongation of fatigue–induced increase in relaxation time can also be attributed to the increased Ca^{++} entry.

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