EXPERIMENTAL MODEL FOR *IN VIVO* TESTING OF MUSCLE RELAXANT DRUGS

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Abstract. The transversal deformation response force is a useful parameter for *in vivo* evaluation of skeletal muscle contractile response in basal conditions, electrical stimulation (induced tetany) or after myorelaxing drugs administration. The present device allows the recording of the transversal deformation response force which represents the parameter for the evaluation of skeletal muscle tone. The purpose of this study was to develop an experimental device suitable to evaluate the *in vivo* relaxing effects of a new purified *Helleborus purpurascens* root extract (ACSw05) on skeletal muscle tone. In this study we used Sprague Dawley rats (200–250 g), that were immobilized on the contention table, after they had been anesthetized with Nembutal 20 mg/kg i.p. Tetany was induced by supramaximal (120%) electrical stimulation. The evaluation of ACSw05 extract effects on skeletal muscle tone was performed by analyzing the plotted length-force curves; the follow-up period was 90 minutes. First we performed a control recording curve and afterwards we injected intramuscular 200 µl ACSw05 extract. We noticed a decrease of length-force curve slope and a 40% decrease of the contractile muscle response compared with the control recording curve. The relaxation curve slope has not shown significant changes compared to the control recording curve. The device and method for skeletal muscle tone evaluation by measuring the transversal deformation response force can be used to compare the effects of different physical, chemical factors and drugs on the skeletal muscle.

Key words: skeletal muscle, muscle tone, contractile response, myorelaxing drugs.

INTRODUCTION

Muscle tone represents an important parameter in studying the physiology, pathology and rehabilitation. In different situations, like muscular dysfunction, normal daily activity or exercise, the muscle tone can be greatly influenced. Physiotherapists examine the status of skeletal muscle tone by palpation. According to this, different methods for quantitative palpation are developed [1, 3, 4, 6].

Received: August 2008;
in final form November 2008.

ROMANIAN J. BIOPHYS., Vol. 18, No. 4, P. 317–327, BUCHAREST, 2008

Fisher reported that in different conditions, such as muscle cramps, edema and spasms the muscle became stiffer [4]. Following a clinical trial, Sakai *et al*. used different techniques to evaluate the muscle tone for cranial musculature, on patients with headache induced by increased blood pressure [11]. According to these studies, patients with headache induced by increased blood pressure presented a significantly increased muscle tone for the trapezium muscle, comparatively to normal patients (indemnifed subjects). Following an ergonomic study, Horikawa [6] reported that the subjects that work on video displays terminals have an increased muscle tone for cranial muscles.

It is well known that the muscles become stiffer during contraction, or in other words, the muscle tissue increases its tone during contraction. The muscle tone increases while its passive or/and active tension increases.

This means that measuring of muscle tone can be used as a general index for physiological and pathological status of skeletal muscle. These measurements can be also used for testing the different pharmacodynamic effects of compounds that act on skeletal muscles.

MATERIALS AND METHODS

The experimental model is realized using Sprague Dawley rats weighting 200–250 g. All experiments were carried out according to procedures approved by the Animal Ethics Committee of University of Medicine and Pharmaceutics Timişoara.

To evaluate muscle tone a method is used for recording the response force (tension), which appears during transversal deformation of the skeletal musculature. The setup used to measure the response tension during deformation is presented in Fig. 1. The progressive deformation of the muscle is realized using a linear moving system Hugo Sachs Vernier Control 850EM, powered by an electric engine (M). On the vernier (V) a Hugo Sachs K300 tension transducer (TT), able to measure a maximum tension of ±300 gf, was fixed. The tension transducer is equipped with a pressure rod (TP), with a diameter of 8 mm, used to apply the force that induces the transversal deformation on the muscle where the tone is measured.

The response tension of the muscle towards deformation which is proportional with the muscle tone is measured by the isometric tension transducer. Progressive deformation of the muscle is induced by the linear movement of the tension transducer and the pressure rod, with the help of the vernier (V). The deformation, which determines the progressive elongation of tested muscle, is measured by using a Hugo Sachs B40 movement transducer (MT). All data were digitized with a sampling rate of 5 ms using an A/D converter and a data acquisition system Axon Digidata 1200 A. The signals are recorded and analyzed with Axon Instruments Axoscope 9.

Passive muscle tension is measured during deformation and it can be plotted as length-tension characteristic curve.

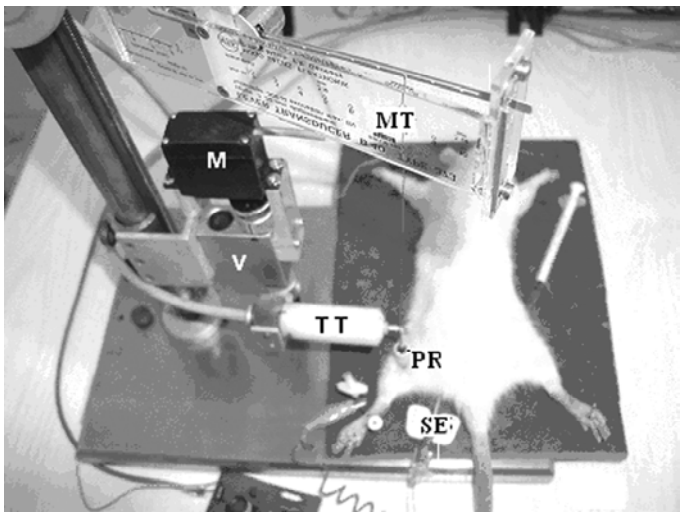

Fig. 1. The setup for recording muscle tone by measuring isometric tension in the skeletal muscle during the elongation and recovery of the muscle to the initial length. (MT = movement transducer; M = electric engine for linear movement; V= vernier for linear movement of the isometric tension transducer and pressure rod; TT = isometric tension transducer; PR = pressure rod; SE = stimulating electrodes).

Two experimental models are used to evaluate the effects of different drugs on skeletal muscle tone. The anesthesia used in both cases consisted of an intraperitoneal injection of Nembutal 20 mg/kg. The anesthetized rat is immobilized on a contention table by binding the limbs on four mobile supports. The experiments are performed at room temperature of 20 °C.

Experimental model 1 evaluates muscle tone under basal conditions (passive muscle tension). The soleus muscle is deformed by applying, in the central part, a pressure, perpendicular to muscle fibers. Measuring the degree of transversal deformation of the muscle allows the quantification of muscle fiber elongation. Simultaneous measuring of the reaction force of the muscle which appears during progressive elongation (passive tension) permits the noninvasive measuring of length-tension characteristic curve. Passive length-tension characteristic curve can be evaluated during both progressive elongation of the muscle and recovery to its initial length (Fig. 2).

Passive muscle tone (the responsive force to the transversal deformation pressure) increases during progressive elongation (ascendant phase) and decreases during the recovery to the initial muscle length (descendant phase). A hysteresis phenomenon is observed during the descendent phase. Specifically, the responsive force of the muscle correlates with the passive tone, with no effect on hysteresis.

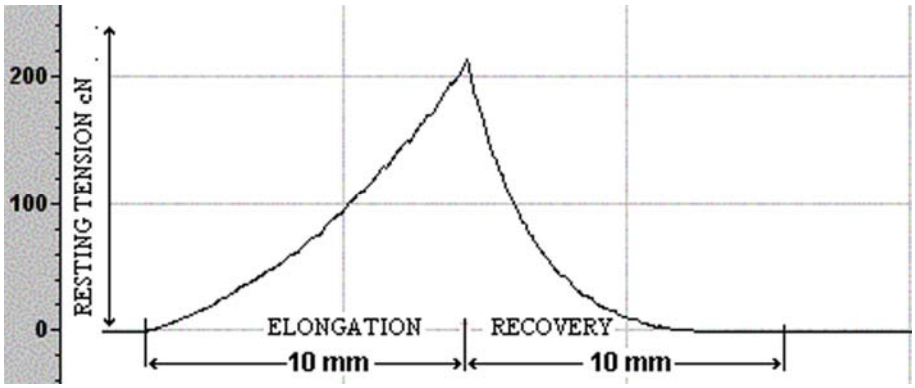

Fig. 2. Measuring length-tension characteristic on the soleus muscle of the rat during both elongation of the muscle (ascendant phase) and the recovery to the initial muscle length (descendant phase). Because of the hysteresis that appears during the descendant phase, a difference between the two curves is noticed.

Experimental model 2 evaluates the muscle tone during contraction. The contracture status is induced by stimulating the muscle using an electric pulse train with the pulse width of 2 ms, frequency of 30 Hz and an intensity of 10 fold threshold level. On this experimental model, simultaneous measuring of reactive force of the muscle towards transversal deformation (tension), and the elongation degree of the muscle (muscle length), allows plotting of the length-tension characteristic curve.

RESULTS

In order to be able to differentiate the effects of anesthesia on basal muscle tone, from the effects of the muscle relaxant drug, we observed the evolution of basal muscle tone in anesthetized rats for 90 minutes after complete anesthesia. Recordings are done using the setup presented in Figure 1. A typical recording for the relation between progressive muscle elongation (transversal deformation) and passive isometric tension (responsive force), in the absence of any voluntary muscle contraction (basal conditions), is presented in Figure 3. A transversal deformation of 5 mm in vertical plane applied to the muscle leads to an exponential increase of the passive tension. A length-tension characteristic curve can be plotted according to the pairs of values representing the elongation degree of the muscle and passive isometric tension developed as a reaction to elongation. Length-tension characteristic curve defines at its best the muscle tone and its reciprocal, the compliance.

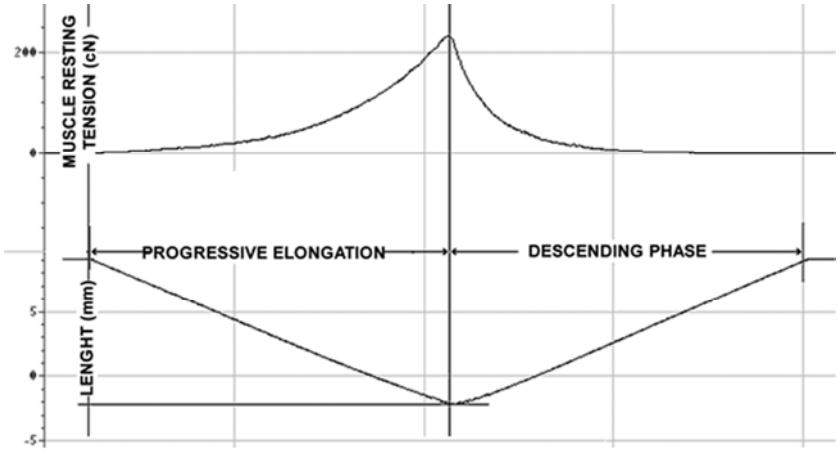

Fig. 3. Simultaneous recording of passive tension and elongation of muscle fibers. The recording is used to plot the length-tension characteristic curve in order to evaluate muscle tone.

The results presented in Table 1 show that for a period of 90 minutes after complete anesthesia, passive isometric tension, developed during progressive elongation of the muscle (muscle tone), presents a slow decrease.

Table 1

Basal tone evolution (cN) during 90 minutes anesthesia

Elongation (mm)	Recording time (min)										Mean value	SD
	Control	10	20	30	40	50	60	70	80	90		
0	0	0	0	0	0	0	0	0	0	0	0	0
0.5	0.20	0.25	0.18	0.25	0.21	0.27	0.22	0.23	0.23	0.24	0.23	0.02
1	1.67	1.86	1.97	1.87	1.87	1.62	1.89	1.69	1.68	1.78	1.79	0.11
1.5	4.74	4.72	3.53	3.74	3.84	3.71	3.79	3.91	3.94	3.87	3.98	0.39
2	9.06	9.41	8.22	6.77	6.19	6.08	7.21	8.40	9.10	8.30	7.87	1.16
2.5	16.1	16.94	16.31	15.9	15.4	16.37	16.27	16.31	16.51	15.53	16.16	0.44
3	28.87	28.72	27.78	26.1	26.8	27.32	26.22	26.95	27.27	27.43	27.35	0.87
3.5	52.71	51.98	49.25	49.5	49.8	48.18	49.29	49.69	48.93	48.17	49.74	1.41
4	100	98.56	97.83	97.6	97.5	97.15	96.83	96.78	97.12	96.84	97.63	0.94

The decrease is less than 4% of the control value represented by isometric passive tension measured immediately after complete anesthesia. All measurements performed for testing the effects of muscle relaxant drugs are corrected taking into consideration the spontaneous decrease of passive isometric tension that happens during the 90 minutes of testing. Mean values measured under previous conditions serve as a reference (control) for evaluation of the effects of muscle relaxant drugs tested.

These recordings show that the decrease of basal tone produced by anesthesia during the whole period of 90 minutes of testing is less than 4% (Table 1 and Figure 4).

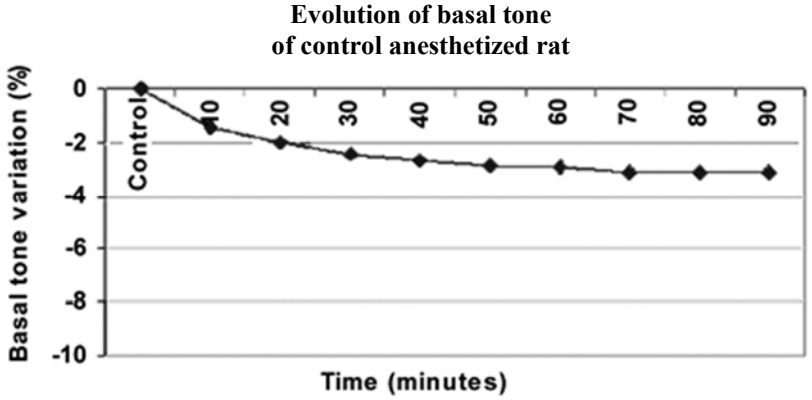

Fig. 4. Basal muscle tone evolution in anesthetized rats. No pharmacodynamic drugs active on skeletal muscle are administered.

"ACSw05" EFFECTS ON BASAL MUSCLE TONE

Intramuscular administration of ACSw05 10 μg/kg, on anesthetized rats, produces a progressive decrease of the passive isometric tension (cN) developed as a reaction to progressive elongation of the muscle. The elongation range is 0.5–4 mm (Table 2).

Table 2

Basal muscle tone (cN) in anesthetized rat after ACSw05 compound (10 μg/kg) administration

Elongation (mm)	Recording time after ACSw05 compound administration (min)									
	Control	10	20	30	40	50	60	70	80	90
0.5	0.18	0.24	0.15	0.22	0.18	0.07	0.19	0.12	0.11	0.13
1.0	1.49	1.67	0.87	0.78	0.78	0.55	0.80	1.16	1.51	0.96
1.5	4.24	4.23	3.77	3.77	3.49	3.2	3.19	3.63	4.10	3.47
2.0	8.12	8.43	7.36	7.2	7.02	6.61	6.46	6.89	6.60	6.6
2.5	14.42	15.17	13.71	11.55	13.1	12.7	12.78	13.71	14.79	13.90
3.0	27.85	27.52	26.7	24.9	24.14	22.6	22.59	24.14	24.9	25.8
3.5	49.8	48.9	45.7	46.33	44.58	42.5	42.37	41.20	42.04	44.05
4.0	89.57	87.74	84.6	83.3	82.71	78.3	76.6	75.47	76.21	79.14

The analysis of data presented in Table 2 shows that the maximum sensitivity in evaluating muscle relaxant effect is obtained at a transversal muscle deformation (elongation) of 4 mm, where the maximum passive isometric tension is obtained. The time-effect curve for the tested drug (ACSw05 10 µg/kg) is drawn according to the values of the passive isometric tension measured at a transversal muscle deformation of 4 mm (Table 3).

Table 3

Percentage modification of passive isometric tension after ACSw05 compound (10 µg/kg) administration

Time (min)	Control	10	20	30	40	50	60	70	80	90
Muscle tone (cN)	89.57	87.74	84.6	83.3	82.71	78.3	76.6	75.47	76.02	79.12
Modify (%)	100%	97.95	94.44	92.99	92.33	87.41	85.51	84.26	84.86	88.32
Variation (%)	0	–2.05	–5.56	–7.01	–7.67	–12.59	–14.49	–15.74	–15.14	–11.68

The data presented in Table 3 prove that the maximum effect consists in a 15.74% decrease of the basal muscle tone from the control value of the passive isometric tension, measured before ACSw05 administration. According to these data a characteristic time-effect curve can be drawn (Fig. 5).

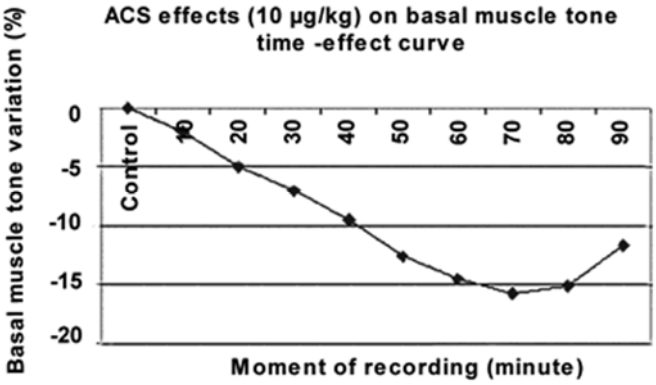

Fig. 5. Time-effect curve of the ACSw05 on skeletal muscle basal tone after a single dose of 10 µg/kg in anesthetized rat.

The analysis of the time-effect curve shows that the muscle relaxant effect of the tested drug (ACSw05 10 µg/kg) becomes evident after 20 minutes from drug administration (5.56%). After this moment the effect progressively increases till 70 minutes after drug administration when it reaches the maximum value of 15.74%. After this moment the effect starts to decrease and at 90 minutes after a drug

administration it becomes 11.68 %. The evolution of the muscle relaxant effect in time of the tested drug (ACSw05 10 μg/kg) after a single dose administration is presented in Figure 5.

EFFECT OF THE ACSw05 ON THE MUSCLE, DURING TETANIC CONTRACTION

These tests are made using the experimental model 2 that allows the evaluation of the muscle tone during tetanic contraction. The tetanic contraction is produced with electric stimulation using a train of 2 ms pulses with a frequency of 30 Hz and an intensity of 10 fold threshold level.

Table 4 shows the control values and the time evolution of the passive isometric tension in the soleus muscle during tetanic contraction, after intramuscular administration of a single dose of 10 μg/kg ACSw05.

Table 4

Passive isometric tension (cN) of the skeletal muscle during tetanic contraction after ACSw05 compound (10 μg/kg) administration

Elongation (mm)		Recording time (min)								
	Control	10	20	30	40	50	60	70	80	90
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	1.05	0.64	0.28	0.25	0.34	0.24	0.15	0.20	0.17	0.18
1.0	1.79	1.56	1.28	0.79	0.83	0.56	0.30	0.41	0.31	0.40
1.5	4.17	3.07	2.12	1.59	1.79	0.95	0.45	0.48	0.49	0.48
2.0	6.59	4.54	3.59	2.61	2.38	2.17	1.03	1.19	0.67	0.73
2.5	8.98	6.18	1.65	3.63	3.53	1.93	1.65	1.81	1.34	1.78
3.0	10.43	7.30	5.82	5.37	5.14	3.00	2.72	2.39	1.84	2.09
3.5	12.39	8.59	6.93	5.45	7.55	4.22	4.39	3.58	2.58	3.49
4.0	15.29	10.87	9.17	7.61	9.45	5.78	5.71	4.36	4.01	4.59
4.5	19.45	12.74	10.95	11.18	11.58	7.33	6.54	5.91	5.26	5.79
5.0	23.67	16.19	13.29	12.60	13.81	10.23	9.14	9.37	9.21	9.48
5.5	27.84	20.49	15.47	16.34	15.30	13.75	10.97	11.71	10.84	11.89
6.0	32.61	25.54	20.66	19.04	20.28	16.67	14.14	13.90	13.06	14.62
6.5	40.15	32.02	27.13	24.85	23.27	20.61	18.09	16.01	15.11	17.98
7.0	49.86	40.47	36.52	31.18	28.57	25.91	22.87	22.78	19.54	23.41
7.5	61.24	49.32	42.38	36.59	34.60	30.81	27.73	27.03	26.14	27.52
8.0	73.67	61.81	54.52	48.32	43.53	43.29	36.91	35.75	34.06	36.06
8.5	90.06	74.50	64.91	60.95	54.99	50.68	46.44	44.09	43.15	45.75
9.0	108.5	94.27	81.93	75.45	68.47	66.40	56.47	54.39	54.13	56.25
9.5	130.5	113.4	101.3	94.90	91.38	82.27	76.18	74.07	73.07	76.89
10.0	157.4	142.9	131.7	120.3	116.4	105.2	100.1	96.55	93.86	98.36

The effect is observed during the first 10 minutes. The evolution of the ACSw05 effect (10 μg/kg) produced by a single dose administration is presented as a time-effect characteristic curve in Figure 6.

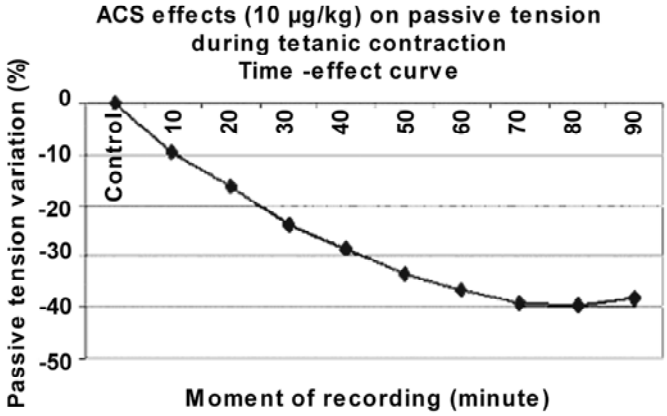

Fig. 6. Time-effect curve produced by the skeletal muscle during tetanic contraction after a single dose of ACSw05 (10 µg/kg).

The analysis of these data shows that ACSw05 produces a marked decrease of the passive isometric tension in skeletal muscles during tetanic contraction. The effect is observed in the first 10 minutes and increases progressively till 80 minutes after drug administration. After this moment the effect decreases.

DISCUSSION

Generally, if a string is tautened by tensile force, it becomes stiffer, and the resistance causing filliping or pushing also becomes larger. Similarly, the tension change in the muscles should be caused by this string-like property.

In the present study, it was reasonable that the muscle tone of the soleus muscle increased with increasing muscle tension because the muscle ends are attached to the bones in the body, in the same way as a string.

It is known that, when the muscle is stretched, from its initial length, the parallel elastic component has an effect upon passive tension. Biophysical studies show that a skeletal muscle giant structural protein (connectine-titine) plays an important role in passive tension [5, 10]. On the other hand, during descendent phase the muscle returns to its initial length. Thus, the tension decreases as a result of the viscous-elastic effect. This effect is related to the connective tissue properties [12]. In conclusion, the first experimental model allows the quantification of the pharmacodynamic effects on basal muscle tone, in the absence of muscular voluntary contraction.

Komiya *et al.* measured muscle tone using a pressure method during voluntary contraction of the forearm muscles [9]. They observed a linear relation between muscle tone and active contractile tension. Although tetanic contraction

induced by the electrical stimulation is not similar to voluntary contraction, a linear increase of the reactive tension during tetanic contraction shows that the effects on muscle tone are not significantly different. During muscle contraction, dense filaments are stabilized by connectine-titine. Contraction isometric tension is produced by the actine-myosin interactions [7]. It has been also observed that muscle stiffness during contraction was related with the degree of interactions between actine-myosin bridges, because stiffness changes were accompanied by modifications of the electromyogram [8]. Moreover, after mechanomyogram evaluation, transversal mechanical properties of the entire muscle mass proved to be a function of certain muscle parameters (muscle length, muscle mass, Young's elastic modulus) [2].

Our data show that this experimental model, by measuring the transversal deformation response force, is proper for emphasizing pharmacodynamic effects of muscle relaxant drugs on the muscle tone that appears during the contracture status.

CONCLUSION

In conclusion, muscle tone modification, when evaluated as the response force, should be accompanied by changes in the passive and active muscle tension, as shown in this experiment.

On the basis of these results it can be assumed that the muscle tension change induced by fiber damage or structural change has also an effect on muscle tone change.

The device and method allow the reproducible and accurate evaluation method of the transversal deformation response force, both in physiological and dysfunctional muscle state, and it can also be used to compare the effects of different physical, chemical factors and drugs on the skeletal muscle.

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