Palestrica of the third millennium – Civilization and Sport
Vol. 14, no. 3, July-September 2013, 181–185

The effect of amlodipine on motility and muscle tonicity
Efectul amlodipinei asupra motilităţii şi tonusului muscular

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Abstract
Background. Hypertension is the most common cardiovascular condition in adults. It is also very common in athletes. In addition to lifestyle changes, medications may be needed for the treatment of hypertension. Medication treatment can be complicated because of restrictions by athletic organizations and possible limitations on maximal exercise performance.
Aims. First-line therapy for athletes and physically active individuals may be different from that of the general population. Dihydropyridine calcium channel blockers (CCBs) are a reasonable choice. Despite their effects on heart rate, non-dihydropyridine CCBs do not appear to impair exercise performance. Treatments in active individuals are recommended in order to allow the best competitive sports results and reduce cardiovascular risk.
Methods. The experiments were conducted on white male Wistar rats. Substances were administered intraperitoneally. The groups were divided according to the substance used as follows: untreated control, amlodipine in 3 doses, reference substances (with a known action) in 1-2 doses, solvent. The tests used were: the open field test studying motility, curiosity, emotions in a new environment, and the recovery test exploring muscle tone on a rigid bar. For statistical analysis, we used the non-parametric chi-square test.
Results. Amlodipine increased motility to a mean value of 74.50±5.48 at a dose of 1.25 mg/kg, but motility decreased with the increasing dose. Significant differences in motility occurred under the influence of amlodipine at a dose of 1.25 mg/kg (p=0.002, t=4.05), and 5 mg/kg (p=0.004, t=-2.37). There were also significant differences in motility between the doses of 1.25 and 2.5 mg/kg (p=0.002, t=4.24) and 5 mg/kg (p=0.0003, t=5.39).
Conclusions. Amlodipine significantly increased motility in a dose of 1.25 mg/kg (p=0.002) and significantly decreased it in a dose of 5 mg/kg (p=0.04). There were no significant changes in muscle tone.
Key words: amlodipine, motility, muscle tone.

Rezumat
Premize. Hipertensiunea este cea mai frecventă afecţiune cardiovasculară la adulţi. De asemenea, este frecventă şi la atleţi. În afara schimbării stilului de viaţă, trebuie început tratamentul medicamentos al hipertensiunii. Tratamentul poate fi complicat datorită restricţiilor impuse de Federaţia de atletism şi poate limita performanţele sportive.
Obiective. Terapia de primă linie la atleţi şi persoane fizic active trebuie diferenţiată de cea a populaţiei generale. Dihidropiridinele - blocante ale canalelor de calciu (CCB) sunt o alegere rezonabilă. În ciuda efectului asupra frecvenţei cardiace, nondihidropiridinele CCB nu influenţează performanţa sportivă. Tratamentul recomandat trebuie să dea cele mai bune rezultate sportive şi reducerea riscului cardiovascular.
Metode. Experimentele s-au efectuat pe şobolani masculi, rasa Wistar. Substanţele au fost administrate intraperitoneal. Loturile au fost împărţite în funcţie de substanţele folosite în: grup netratat, amlodipine în 3 doze, substanţe de referinţă (cu acţiune cunoscută) în 1-2 doze, solventul. Testele folosite au fost: testul Openfield, care studiază motilitatea, curiozitatea şi emotivitatea într-un mediu nou şi testul de redresare, care explorează tonusul muscular pe o bară rigidă. Analiza statistică s-a efectuat cu testul nonparametric chi.
Rezultate. Amlodipina creşte motilitatea la o medie de 74,50±5,48 la doza de 1,25 mg/kg, dar motilitatea scade cu creşterea dozei. Diferenţa semnificativă s-a obţinut sub influenţa amlodipinei pe motilitate la doza de 1,25 mg/kg (p=0,002, t=4,05), şi la doza de 5 mg/kg (p=0,004, t=-2,37). Diferenţe semnificative s-au obţinut pe motilitate între dozele de 1,25 şi 2,5 mg/kg (p=0,002, t=4,24) şi 5 mg/kg (p=0,0003, t=5,39).
Concluzii. Amlodipina creşte semnificativ motilitatea la doza de 1,25 mg/kg (p=0,002) şi scade semnificativ la doza de 5 mg/kg (p=0,04). Asupra tonusului muscular nu s-au înregistrat modificări semnificative.
Cuvinte cheie: amlodipina, motilitate, tonus muscular.

Received: 2013, May 5; *Accepted for publication:* 2013, June 18;
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Introduction

Hypertension is the most frequent cardiovascular disorder in adults. Physically active individuals and professional athletes are also affected by hypertension. This is extremely common among athletes, decreasing life expectancy and generating substantial costs for the health care system. Although the proportion of affected individuals is significantly lower compared to the rest of the population, these should be constantly evaluated and monitored for blood pressure, in order to ensure competitive and safe sports participation (Asplund, 2010).

Regarding treatment, the implementation of changes in the lifestyle will be the routine in athletes and active individuals, having the same importance as for the rest of the population.

When lifestyle does not change, drug treatment should be administered for hypertension. When choosing the antihypertensive preparation, producers should select a preparation with favorable effects on blood pressure, as well as minimal hemodynamic changes during exercise. Drug treatment may be complicated because of restrictions imposed by sports organizations, and it can also diminish maximal sports performance. When pharmacological therapy is indicated in physically active persons, this will be ideal when: a) blood pressure is low at rest and during exercise; b) total peripheral resistance decreases, and c) it has no adverse effects on exercise capacity.

For these reasons, angiotensin converting ezyme inhibitors (ACEIs) and angiotensin II receptor blockers (in case of intolerance to angiotensin inhibitors) and calcium channel blockers are the drugs of choice in the case of mild exercise and in athletes with primary hypertension (Pescatello et al., 2004; Oliveira & Lawless, 2010).

In addition, antihypertensive drugs will be chosen taking into consideration the water and salt losses that usually occur in athletes, as well as the maintenance of sports performance and endothelial function. The effects of diuretics are less desirable and non-selective beta-blockers will be the last choice in hypertensive patients that are physically active (Asplund, 2010; Fagard, 2011).

First-line therapy in athletes may be different from that in the general population. Dihydropyridines are another reasonable choice. In spite of their effects on heart rate, dihydropyridines do not seem to affect sports performance (Fagard, 2011).

Calcium channel blockers inhibit slow calcium channels, reducing in this way calcium concentration in vascular smooth muscle cells, which results in a decrease of vascular and systemic resistance and general vasodilation. The effects of calcium channel blockers depend on the depolarization time, dose and chemical composition of the drug (Godfrain, 1989).

Dihydropyridines are L-type calcium channel blockers (Godfrain, 1987). Compared to them, phenylalkylamines (e.g. verapamil) have a more important action on the heart. Dihydropyridines are relatively vascular selective through their action mechanism in decreasing blood pressure. Medication with the dihydropyridine class that selectively acts on L-type channels occurs through the allosteric alteration of the channel gate. Due to the lipophilic properties of dihydropyridines, type L calcium channel blockers cross the blood-brain barrier (e.g. felodipine, isradipine, nicardipine, nifedipine, nimodipine, nitrendipine, lacidipine, lercandipine), while a dihydropyridine apparently does not cross the blood-brain barrier (amlodipine) (Ritz et al., 2010).

This process may influence the oxygen and nutrient supplementation of the skeletal muscles and it also plays a role in removing catabolites.

Objectives

To evaluate the effect of amlodipine, a dihydropyridine compound, on the motility and muscle tone of rats, in order to evidence the presence of significant adverse effects on physical performance.

Hypothesis

Amlodipine treatment will improve exercise, compared to the control group.

Material and methods

Research protocol

a) *Period and place of the research*

All the animals used in this study were kept under accredited conditions and the described experiments were carried out according to the 1986 Directive of the European Committee (86/609/EEC) and Ordinance no. 37 of the Romanian Government of 2 February 2002.

b) *Subjects and groups*

The experiments were performed on white male Wistar rats with a weight of 125±25 g. The animals were fed with standard laboratory food and received water ad libitum (Beiderbeck et al., 2012). The study groups were as follows: three groups treated intraperitoneally with different amlodipine doses (Pfizer Mack GmbH) (1.25, 2.5, 5 mg/kg body weight) diluted in 1 ml propylene glycol; an untreated negative control group; a control group treated intraperitoneally with 1 ml propylene glycol (Farmec S.A.) used as a solvent for amlodipine; two groups treated intraperitoneally with haloperidol (Schering-Pflough) (0.25, 0.5 mg/kg body weight). The doses were calculated at 1/10 of LD 50. The doses were chosen based on the acute toxicity of these preparations administered to rats by intraperitoneal route (Danilă et al., 1984). The reference substances were chosen depending on the test.

c) *Tests applied*

The open field test studies the motility, curiosity and emotions of animals in a new environment. The open field test was applied for three minutes to each animal separately. The mean of the crossings from one sector to another and the rears represent the spontaneous motility score (Rainer, 2003; Neumann, 2011).

The traction test explores muscle contraction in the animals using the Rotarod test and the evasion test on an inclined plane.

The recovery test evaluates muscle tone on a rigid bar (Matsumoto et al., 2002).

d) *Statistical processing*

All arithmetic means, standard deviations, standard errors and statistical significances were calculated

according to the Student test. Statistical processing was performed for the multivariate analysis of the variance with the Student t test. The probability value chosen was a p threshold of 0.05, with a significant value. The data were analyzed using SPSS version 11 for Windows.

For the recovery test, the non-parametric chi square test was used.

Results

The main tested substance was amlodipine.

Motility was tested in the open field in naïve animals. The results obtained were introduced in tables and figures.

Amlodipine increased *motility* compared to propylene glycol to a mean score of 74.50±5.48 in a dose of 1.25 mg/kg; motility decreased with the increase of the dose. Thus, at a dose of 2.5 mg/kg, a mean score of 32.67±8.19 was found, and at 5 mg/kg, the mean score was 25.67±7.20. Propylene glycol had a mean score of 45.83±4.50, and diazepam approximately the same mean of 50.70±6.30 (Table I, Fig. 1).

Table I

Motility score under the influence of amlodipine.

Indicator	Control	Propylene glycol	Amlodip 1.25 mg	Amlodip 2.5 mg	Amlodip 5 mg	Diazepam
Mean	55.1	45.833	74.5	32.667	25.667	50.7
Std. dev.	20.442	120.967	13.428	20.057	311.467	19.945
Std. error	6.46	10.998	5.482	8.188	17.648	6.307

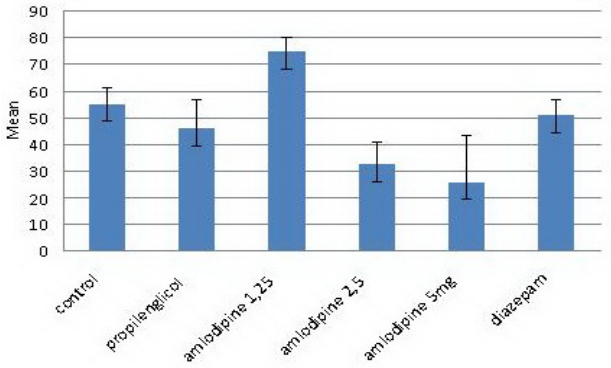

Fig. 1 – Motility score under the influence of amlodipine.

The statistical processing of data shows that motility was not significantly influenced by propylene glycol, the solvent of amlodipine.

Table II

"p" variation of motility under the influence of amlodipine.

Substances	Mean difference	t	p
Amlo - 1.25, Amlo - 2.5	41.833	4.245	.0017
Amlo - 1.25, Amlo - 5	48.833	5.394	.0003
Amlo - 1.25, Lact -	41.167	5.224	.0004
Amlo - 1.25, Pg+Sf -	28.667	4.046	.0023
Amlo - 2.5, Amlo - 5	7.000	.642	.5354
Amlo - 2.5, Lact -	-.667	-.067	.9479
Amlo - 2.5, Pg+Sf -	-13.167	-1.410	.1889
Amlo - 5, Lact -	-7.667	-.837	.4223
Amlo - 5, Pg+Sf -	-20.167	-2.375	.0389
Lact - , Pg+Sf -	-12.500	-1.730	.1143

There were significant differences in motility under the influence of amlodipine compared to propylene glycol at a dose of 1.25 mg/kg (p=0.002; t=4.05), and at a dose of 5 mg/kg (p=0.004; t=-2.37). Also, there were significant differences between the doses of 1.25 and 2.5 mg/kg (p=0.002; t=4.24) and 5 mg/kg (p=0.0003; t=5.39). A comparison of the effects of the other doses on motility shows no significant differences (Table II, Fig. 2).

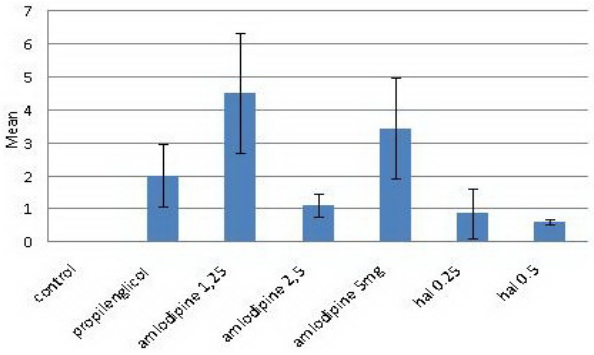

Fig. 2 – Muscle tone score under the influence of amlodipine.

The influence of propylene glycol on the rears was expressed by a mean score of 6.00+1.033, which increased under the action of amlodipine in a dose of 1.25 mg/kg to 12.67+1.085, and then decreased to 4.167+1.075 at a dose of 2.5 mg/kg and to 2.333+0.76 at a dose of 5 mg/kg (Table III).

Table III

Rearing score under the influence of amlodipine.

Indicator	Propylene glycol	Amlodip 1.25 mg	Amlodip 2.5 mg	Amlodip 5 mg
Mean	6	12.67	4.167	2.333
Std. dev.	2.53	2.66	2.64	1.86
Std. error	1.033	1.085	1.075	0.76

There were significant differences in rears between the control group (propylene glycol) and the group with amlodipine in a dose of 1.25 mg/kg (p-0.001; t-4.45), and the group with a dose of 5 mg/kg (p-0.01; t- -2.86). There were also significant differences between the groups with different amlodipine doses; thus, between the dose of 1.25 mg/kg and the dose of 2.5 mg/kg p-0.0002; t-5.56, and between the amlodipine dose of 1.25 mg/kg and 5 mg/kg the probability was p<0.0001; t-7.80. There were no statistically significant differences between the other groups (Table IV).

Table IV

"p" variation under the influence of amlodipine on rears.

Substances	Mean difference	t	p
Amlo - 1.25, Amlo - 2.5	8.500	5.558	.0002
Amlo - 1.25, Amlo - 5	10.333	7.799	<.0001
Amlo - 1.25, Lact -	5.667	1.545	.1533
Amlo - 1.25, Pg+Sf -	6.667	4.450	.0012
Amlo - 2.5, Amlo - 5	1.833	1.390	.1946
Amlo - 2.5, Lact -	-2.833	-.773	.4573
Amlo - 2.5, Pg+Sf -	-1.833	-1.228	.2475
Amlo - 5, Lact -	-4.667	11.302	.2221
Amlo - 5, Pg+Sf -	-3.667	-2.859	.0170
Lact - , Pg+Sf -	1.000	.274	.7898

The mean time of recovery on the rigid bar was 2±0.95 seconds for propylene glycol, and under the influence of amlodipine at a dose of 1.25 mg/kg it increased to 4.5±1.80 seconds, decreased at the dose of 2.5 mg/kg to 1.08±0.33, but increased at the dose of 5 mg/kg to 3.42. Under the action of haloperidol, the recovery time decreased to a mean time of 0.83±0.75 for the dose of 0.25 mg/kg and to 0.58±0.08 for the dose of 0.5 mg/kg (Table V).

Table V
Statistical values of recovery on the rigid bar under the influence of amlodipine.

Group	Mean	Standard deviation	Standard error
Propylene glycol	2	2.32	0.95
Amlodipine 1.25 mg	4.5	4.40	1.80
Amlodipine 2.5 mg	1.08	0.80	0.33
Amlodipine 5 mg	3.42	3.79	1.55
Haloperidol 0.25 mg	0.83	0.31	0.75
Haloperidol 0.5 mg	0.58	0.20	0.08

There were significant differences only between the reference group compared to the untreated control group (p-0.003; t-2.23). There were no significant differences in the case of the other comparisons by groups.

The proportion of the animals that remained on the bar for 3 minutes was 50% for the untreated group and 0% for propylene glycol 0.5 mg/kg and amlodipine 2.5 mg/kg. The percentage increased to 16.67% for the groups with amlodipine in the doses of 1.25 and 5 mg/kg, reaching 66.67% for haloperidol 0.5 mg/kg (Table VI).

Table VI
Percentage of animals remaining on the rigid bar.

3' (180")	Control	Hal 0.25	Hal 0.5	Pg	Amlo-1.25	Amlo-2.5	Amlo-5
%	50.000	16.667	66.667	0.000	16.670	0.000	16.670

Discussion

Calcium channel blockers inhibit the conductance of slow calcium channels, resulting in the reduction of calcium concentration in the vascular smooth muscle cells, which leads to a decrease in systemic vascular resistance with generalized vasodilation (Bellien, 2013). Calcium channel blockers are effective in reversing ventricular hypertrophy.

Dihydropyridines such as amlodipine (Norvasc) and nifedipine (Procardia) may induce reflex tachycardia, fluid retention, and vascular headaches. Non-dihydropyridines such as verapamil (Calan) and diltiazem (Cardizem) may cause the suppression of heart rate, minor discomfort in the case of a high heart rate, decreased left ventricular contractility and constipation (Dorffel, 2004). Calcium channel blockers have no major effects on energy metabolism during exercise, and the maximal oxygen uptake is generally maintained. There is a potential for the competitive mechanism of blood flow steal at muscular level (caused by vasodilation) and the early onset of the lactate threshold. The way in which contracture induced by aggression leads to the increase of calcium remains unknown. However, calcium channel blockers, particularly dihydropyridines, are generally well tolerated and effective in physically active patients. They are frequently used as first-line agents in black athletes (Niedfeldt, 2002).

The endocannabinoid inhibitors at cellular level are capable of exerting a strong action at each of the four tests of the rats on behavioral activities (analgesis on a "hot hob", immobility in a "ring", rectal hypothermia and hypolocomotion in the open field (Ligresti et al., 2006).

Baker (2001) discovered that endocannabinoid inhibitors in the cells have the capacity to inhibit limb spasticity in the rats, with CREAE, a multiple sclerosis (MS) model. This observation was confirmed by the contribution of six other types of inhibitors such as AM404 and arvanil that may also act through TRPV1 receptors (Baker, 2001; Brooks, 2002, Ligresti et al., 2006).

Also, it is known that contractures may cause skeletal muscle lesions that result from ruptures of the protein structure of normal muscles. The contracture that induces muscle lesions is characterized by a series of metabolic events including inflammatory cell infiltration, increase of intracellular calcium concentration, release of muscular enzymes, muscle inflammation, and a marked decrease of voluntary and involuntary strength. The subsequent increase of intracellular calcium concentration as a result of the initial contraction contributes to the progress of the muscle lesion through the stimulation of calcium-activated neutral proteases (CANP) such as calpain (increased calcium affinity and many forms of low calcium affinity). These proteases can initiate proteolysis by cleaving the proteins associated with the "sensitive" Z line such as desmin and actin. The exposure of muscles to other treatments such as calcium ionophores, as well as the increase of intramuscular calcium evidence the same types of morphological and ultrastructural changes as those seen in excentric muscle lesions in the muscle cell. However, one or a combination of the following are plausible reasons: loss of the integrity of the sarcoplasmic reticular membrane; rupture of the sarcolemmal membrane; opening of channels sensitive to stimuli; or the alteration of the triad and the orientation of the t tubes resulting from the entry of calcium through voltage-sensitive channels such as dihydropyridine receptors. Calcium channel blockers and other calcium chelators reduce or prevent contraction, inducing the increase of intracellular calcium levels and subsequent changes following muscle lesions in rodents. The ability of CCB to prevent contraction, induce increases in cytosolic and mitochondrial calcium concentrations and subsequent histological changes indicates that the disturbance of the normal activity of calcium channel blockers is at least partially responsible for allowing calcium to enter the muscle cell (Beaton, 2002).

Conclusions

1. Amlodipine significantly increases motility at a dose of 1.25 mg/kg (p=0.002) and significantly decreases it at a dose of 5 mg/kg (p=0.04).
2. Similarly to its effect on motility, amlodipine at a dose of 1.25 mg/kg increases the number of rears and significantly decreases it at the dose of 5 mg/kg (p=0.01).
3. There are no significant changes in muscle tone.

Conflicts of interest

There are no conflicts of interests.

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