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Contraction force versus action potential duration of cardiomyocytes: which methodology is more informative for selection of effective 1,4-dihydropyridine compounds in experimental research?

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Background. Identification of activity properties of new synthesized compounds is important to help choose the adequate research methodology. The goal of this experimental research was to determine the relationship between the chemical structure of 25 compounds of 1,4-dihydropyridine derivatives and their effects on the contraction force of guinea pig papillary muscles and the action potential (AP) duration.

Methods. AP recordings were obtained with standard microelectrodes that were made from borosilicate glass capillaries, filled with 2.5 M KCl and connected to the high-input impedance amplification system. 1.0 Hz stimulation frequency was used. The contraction of papillary muscles was recorded by using a force transducer. Both signals (inotropic response and AP) were digitised by an A/D converter and registered by a computer specialised program.

Results. The results have shown that most of 1,4-dihydropyridine derivatives possessed the negative inotropic action and had the negligible impact on the AP duration.

It was identified that 1,4-dihydropyridine compound OSI 9719 (2-propoxyethyl-4-difloemethoxyphenyl-2-methyl-5-nitro-1,4-dihydropyridine-3-carboxylate) had a prolonged duration of AP and simultaneously increased the force of contraction ($p < 0.05$).

It was shown that the efficiency of 1,4-dihydropyridine derivatives depends on the nature (diflormethoxy-, propoxy-, ethoxycarbonyl-, amine, atom chlorine, ethylene glycol-, isopropoxy-) of the substituents and their spatial isomerisation in the phenyl and 1,4-dihydropyridine rings and the concentration of the compounds.

Conclusion. Evaluation of the contraction force of guinea pig papillary muscles is a more informative method than evaluation of the action potential duration, conducting experimental research of the activity properties of 1,4-dihydropyridine derivatives.

Key words: 1,4-dihydropyridine derivatives, inotropic action, action potential duration, guinea pig papillary muscles

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INTRODUCTION

Different Ca^{2+} channel antagonists, independently of their chemical structure, block the Cav1.2 L-type calcium channels, decrease the entrance of Ca^{2+} into the cells and support the negative inotropic and vascular relaxing properties. Currently Ca^{2+} antagonists are widely used in the cardiovascular diseases for the prevention and clinical treatment (1). The 1,4-dihydropyridine derivatives, nifedipine and amlodipine [2], possess the greatest selectivity for the vascular voltage gated calcium ions channels (L-type), as Ca^{2+} channel blockers, compared to benzodiazepine diltiazem (3) or phenylalkylamine verapamil (4, 5). The action mechanism of 1,4-dihydropyridines is related to their action on the vascular smooth muscle. The typical resting membrane potential (E_m) of human *vena saphena magna* appeared to be –76.0 ± 7.0 mV at the physiological extracellular K^+ standard and it reaches –64.7 ± 7.0 mV at the 80 mM extracellular K^+ concentration (6). It is supposed that the blocking effect of 1,4-dihydropyridine derivatives on the L-type Ca^{2+} channels should occur at the more negative potential value. Experimental studies confirmed that nifedipine relaxes blood vessels (*v. saphena magna*) maximally at the 20 mM extracellular K^+ concentration which depolarises the membrane potential (E_m) only 4 mV from the resting potential (7). Therefore, the action mechanism of dihydropyridines, among other things, may be attributed to the activation of nitric oxide synthase and NO release from the vascular endothelium (8). Amlodipine is the most studied and widely used in clinical practice third generation agent of 1,4-dihydropyridine derivatives characterized with a significant vascular selectivity and less than nifedipine negative inotropic effects (9). The long elimination half-life and low variability before reaching the peak plasma concentration enable one to obtain a significant therapeutic effect by using once a day (10). Experimental studies have shown that amlodipine reduces blood pressure gradually and at the same time does not cause tachycardia. The prophylactic use of amlodipine protects the cardiomyocytes from the ischemic calcium overload resulting after reperfusion and protects the endothelium from ischemic damage (11). This medicine is relatively well tolerated and has no adverse effects on carbohydrate and lipid metabolism. Further studies have confirmed the hypothesis that the positive effect of amlodipine may be associated not only with a decrease in calcium overload but with the release of nitric oxide from the vascular endothelium (12). However, amlodipine has shown the unwanted side effects as oedema, headache, weakness and dizziness in the long-term clinical studies. Cardiac muscle contractility could be reduced that may lead to the sinus pacemaker and atrioventricular conduction depression (13). These statements were confirmed by experiments with isolated small blood vessels, coronary arteries and aorta. These results have not been derived from studies of the other calcium antagonists, nifedipine and diltiazem (14, 15). The received information from experimental and clinical studies encourages the pharmacologists and chemists for further investigations of amlodipine and newly synthesized 1,4-dihydropyridine derivatives to get more selective and less toxic compounds focusing attention to positive inotropic properties (16, 17).

The goals of this study were the following: (i) to determine the relationship among the chemical structure of 1,4-dihydropyridine derivatives and their effects on the contraction force of guinea pig papillary muscles and the action potential duration; (ii) to highlight what method (contraction force or transmembrane action potential duration) is more informative for the screening of the new synthesized compounds in respect to cardiomyocytes.

MATERIALS AND METHODS

The inotropic activity and transmembrane AP duration (APD) of the presented 1,4-dihydropyridine derivatives were evaluated on the guinea-pig papillary muscles according to the previously described procedures (18). This procedure was reviewed and approved by the Lithuanian State Food and Veterinary Office (Permission to Use the Laboratory Animals in the Research, 29/10/2005, No. 0139). The animals were obtained from the Lithuanian Veterinary Academy (License No. B-76, 06/06/2005).

Briefly, the right ventricle papillary muscles of guinea pig were mounted in an organ bath (volume of 1 ml) with a circulation of Tyrode's solution of the following composition (in mM): NaCl,

144; $CaCl_2$, 1.8; $MgCl_2$, 1; KCl, 4; TrisC1, 10 and glucose, 5; the reaction occurred at pH 7.3–7.4 and 37 °C and was aerated with oxygen continuously. A 1.0 Hz stimulation frequency was used.

Action potential (AP) recordings were obtained with standard microelectrodes that were made from borosilicate glass capillaries (GC150F-10, Harvard Part No. 30-0057, England), filled with 2.5 M KCl and connected to the high-input impedance amplification system. Both signals (inotropic response and AP) were digitised at a minimum sampling rate of 10 kHz with a 12-bit A/D converter (Digidata 1200, Keithley Instruments, Inc., Cleveland, Ohio, USA) and recorded with a computer. The signals were preserved there and analysed to obtain the maximal contraction amplitude and the AP duration at 10, 25, 50 and 90% repolarisation (APD_{10}, APD_{25}, APD_{50} and APD_{90}, respectively). After a 1-h equilibration period, the tested compounds affected each papillary muscle in a dose-dependent manner. Each heart preparation was perfused for 20 min with any concentration.

Drugs

1,4-dihydropyridine derivatives, including amlodipine, were synthesized at the Latvian Institute of Organic Synthesis, Riga, Latvia. For more details about synthesis and various physical and chemical characteristics of the presented 1,4-dihydropyridine derivatives see in (1, 19–22).

Statistical analysis

All the values are presented as means ±SE (Table). A statistical analysis by the Student's t-test was performed for paired and unpaired observation. The level of $p < 0.05$ was adopted as a critical value of significance.

Table. Effect of cumulative doses of 1,4-dihydropyridine derivatives on cardiac action potential duration and contraction force in the isolated papillary muscles of guinea pig hearts

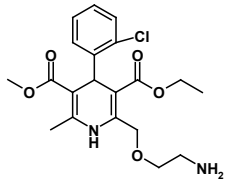
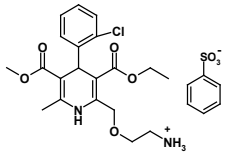
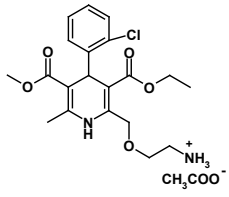
Compound	Dose, mol/l	Contraction force, P/$P_o \pm m_x$, %	Action potential duration, ms			
			APD_{10}	APD_{25}	APD_{50}	APD_{90}
1	2	3	4	5	6	7
 Amlodipine (OSI-9787) (n = 6)	Control	100.0	38.5 ± 1.5	84.7 ± 5.7	125.0 ± 9.3	154.3 ± 12.9
	1×10^{-7}	91.9 ± 6.5	41.5 ± 1.0	88.0 ± 5.2	122.7 ± 10.7	153.0 ± 15.4
	1×10^{-6}	75.4 ± 11.7	37.5 ± 1.8	85.7 ± 9.6	120.7 ± 13.0	153.0 ± 15.3
	1×10^{-5}	60.0 ± 12.7	33.0 ± 2.0	81.0 ± 12.5	117.0 ± 15.0	150.3 ± 13.2
	1×10^{-4}	40.0 ± 5.7	33.25 ± 1.3*	80.0 ± 7.0	114.0 ± 10.8	150.7 ± 12.6
 Norvasc (IOS-9529) (n = 6)	Control	100.0	20.3 ± 3.3	61.8 ± 5.0	102.6 ± 4.2	136.7 ± 6.0
	1×10^{-7}	85.9 ± 6.0	17.9 ± 2.3	55.7 ± 9.2	98.4 ± 5.0	135.3 ± 5.2
	1×10^{-6}	70.7 ± 7.6	18.8 ± 1.8	54.4 ± 5.0	94.3 ± 6.0	133.6 ± 4.2
	1×10^{-5}	55.6 ± 6.3	19.0 ± 2.4	56.7 ± 3.4	102.6 ± 4.6	136.5 ± 7.8
	1×10^{-4}	35.8 ± 3.5	17.7 ± 3.2	56.6 ± 2.8	95.5 ± 2.8	129.4 ± 4.4
 IOS-9528 (n = 5)	Control	100.0	29.2 ± 6.6	71.8 ± 11.7	109.7 ± 11.8	154.5 ± 14.2
	1×10^{-7}	80.56 ± 4.3	26.4 ± 7.3	61.0 ± 14.0	90.5 ± 20.3	136.4 ± 14.0
	1×10^{-6}	64.6 ± 3.4	28.5 ± 5.6	65.2 ± 13.0	98.5 ± 17.4	142.0 ± 13.5
	1×10^{-5}	50.0 ± 1.6	27.5 ± 6.8	63.4 ± 13.7	101.0 ± 14.6	142.8 ± 11.0
	1×10^{-4}	36.6 ± 2.7	32.0 ± 6.8	69.9 ± 11.0	109.0 ± 11.7	146.4 ± 10.0

Table (continued)

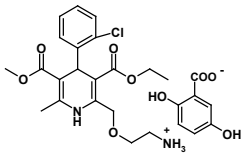
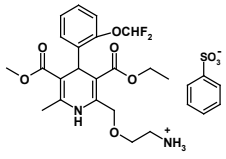
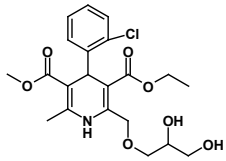
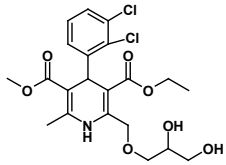
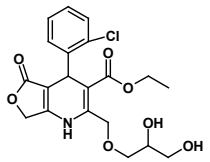
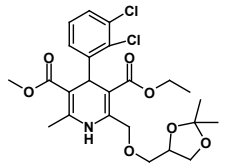
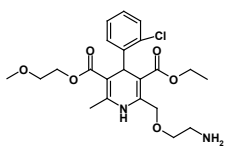
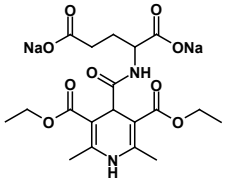
1	2	3	4	5	6	7
	Control	100.0	28.0 ± 6.3	70.0 ± 6.3	111.6 ± 8.5	147.6 ± 7.7
	1×10^{-7}	89.8 ± 3.8	29.75 ± 7.8	68.2 ± 13.4	109.0 ± 11.2	147.5 ± 9.5
	1×10^{-6}	86.8 ± 9.8	30.75 ± 7.15	72.4 ± 10.76	114.8 ± 7.5	150.0 ± 6.7
	1×10^{-5}	76.3 ± 11.9	33.6 ± 7.4	78.6 ± 10.6	121.8 ± 8.8	156.7 ± 7.0
IOS-9587 (n = 5)	1×10^{-4}	60.2 ± 11.0	29.75 ± 7.45	70.5 ± 10.0	112.7 ± 8.8	151.0 ± 6.8
	Control	100.0	28.0 ± 3.6	70.0 ± 2.4	108.0 ± 10.4	139.0 ± 6.2
	1×10^{-7}	77.0 ± 5.6	23.5 ± 2.7	62.5 ± 9.3	99.6 ± 12.0	134.0 ± 8.6
	1×10^{-6}	57.7 ± 9.4	21.0 ± 1.7	57.0 ± 10.2	93.6 ± 14.8	131.2 ± 14.2
	1×10^{-5}	46.4 ± 5.5	20.0 ± 2.1	53.6 ± 9.4	90.5 ± 12.6	127.5 ± 11.5
IOS-9614 (n = 5)	1×10^{-4}	26.7 ± 4.8	17.75 ± 2.4	47.75 ± 8.9	88.0 ± 11.8	132.9 ± 10.7
	Control	100.0	27.6 ± 4.0	69.3 ± 10.8	105.0 ± 15.6	132.0 ± 15.4
	1×10^{-7}	110.4 ± 11.2	26.5 ± 6.2	62.6 ± 13.9	97.7 ± 11.0	134.7 ± 9.4
	1×10^{-6}	105.2 ± 13.8	27.5 ± 4.0	72.3 ± 9.0	111.8 ± 9.9	143.8 ± 8.4
	1×10^{-5}	85.3 ± 17.0	25.2 ± 2.7	70.2 ± 6.8	111.9 ± 7.2	144.6 ± 5.6
IOS-9586 (n = 5)	1×10^{-4}	47.0 ± 8.44	23.7 ± 2.8	60.2 ± 5.0	100.0 ± 6.2	134.5 ± 4.2
	Control	100.0	39.0 ± 2.5	98.0 ± 3.1	140.0 ± 5.6	166.0 ± 8.0
	1×10^{-7}	96.0 ± 8.7	42.3 ± 3.1	100.8 ± 4.1	146.3 ± 7.0	175.6 ± 8.2
	1×10^{-6}	87.0 ± 8.8	41.8 ± 3.3	103.0 ± 4.2	151.0 ± 7.7	180.0 ± 9.0
	1×10^{-5}	71.0 ± 12.3	40.2 ± 4.0	98.3 ± 4.5	148.0 ± 7.5	175.0 ± 8.9
OSI-9599 (n = 5)	1×10^{-4}	49.0 ± 11.2	41.4 ± 2.7	96.4 ± 3.5	141.4 ± 7.5	171.4 ± 7.3
	Control	100.0	39.0 ± 6.6	98.4 ± 8.2	148.0 ± 11.4	182.0 ± 8.6
	1×10^{-7}	85.0 ± 8.7	36.0 ± 2.0	92.4 ± 7.2	141.0 ± 8.0	173.4 ± 7.8
	1×10^{-6}	70.0 ± 12.2	35.0 ± 1.5	91.0 ± 7.2	142.0 ± 7.6	174.0 ± 4.4
	1×10^{-5}	60.0 ± 11.4	36.0 ± 3.0	87.0 ± 6.2	136.0 ± 6.9	166.0 ± 3.8
OSI-9730 (n = 4)	1×10^{-4}	46.7 ± 8.4	38.0 ± 2.8	87.0 ± 5.9	132.5 ± 5.5	165.6 ± 3.1
	Control	100.0	29.5 ± 2.9	71.0 ± 3.4	107.5 ± 3.2	135.2 ± 1.8
	1×10^{-7}	89.0 ± 4.2	26.6 ± 4.85	69.5 ± 3.2	105.2 ± 2.0	136.2 ± 0.9
	1×10^{-6}	67.7 ± 7.7	30.2 ± 4.2	73.6 ± 3.8	110.6 ± 3.5	140.9 ± 1.7
	1×10^{-5}	61.0 ± 10.2	30.0 ± 4.2	74.4 ± 3.8	111.0 ± 3.7	140.0 ± 2.9
IOS-9600 (n = 4)	1×10^{-4}	51.2 ± 10.8	30.75 ± 5.7	76.0 ± 6.6	112.2 ± 2.5	147.0 ± 4.2
	Control	100.0	25.5 ± 6.7	67.5 ± 9.8	113.0 ± 5.0	142.8 ± 2.9
	1×10^{-7}	81.8 ± 6.5	26.3 ± 8.0	68.7 ± 10.3	114.0 ± 6.8	144.0 ± 5.8
	1×10^{-6}	66.8 ± 8.0	28.7 ± 7.8	72.3 ± 8.3	118.0 ± 4.5	152.0 ± 2.3
	1×10^{-5}	50.0 ± 7.7	32.7 ± 8.7	75.0 ± 9.6	119.7 ± 2.6	153.0 ± 0.5
IOS-9597 (n = 4)	1×10^{-4}	30.0 ± 3.0	28.3 ± 6.5	65.0 ± 7.9	103.2 ± 4.0	142.0 ± 0.9
	Control	100.0	25.5 ± 2.8	72.2 ± 4.5	108.6 ± 5.2	150.6 ± 6.6
	1×10^{-7}	94.0 ± 2.7	27.2 ± 2.0	74.0 ± 3.6	115.5 ± 5.0	154.4 ± 7.0
	1×10^{-6}	84.5 ± 3.0	26.0 ± 1.3	72.2 ± 2.8	118.5 ± 3.3	155.5 ± 4.7
	1×10^{-5}	76.3 ± 4.3	25.5 ± 0.9	76.7 ± 6.8	121.5 ± 7.0	158.0 ± 9.0
Gliutapirone	1×10^{-4}	65.0 ± 6.7	24.7 ± 1.2	78.6 ± 9.2	123.0 ± 9.0	160.0 ± 9.5

Table (continued)

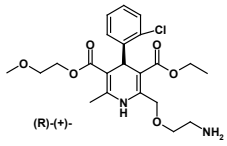
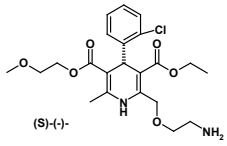
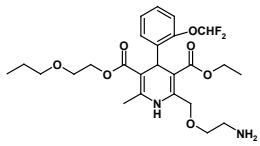
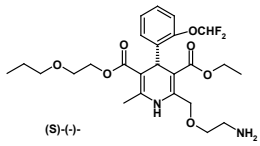
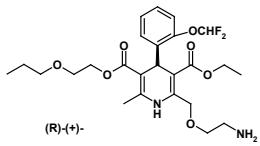
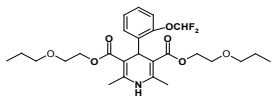
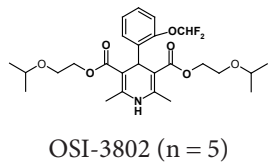
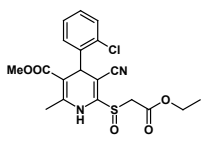
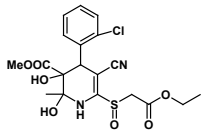
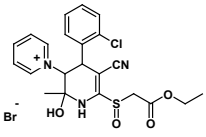
1	2	3	4	5	6	7
	Control	100.0	30.0 ± 6.1	69.7 ± 10.6	110.0 ± 8.5	142.3 ± 6.0
	1×10^{-7}	114.0 ± 24.0	30.0 ± 6.0	75.5 ± 9.0	126.0 ± 6.7	156.0 ± 5.8
	1×10^{-6}	112.9 ± 25.7	31.0 ± 6.8	77.0 ± 4.0	131.7 ± 6.5	162.0 ± 10.2
	1×10^{-5}	99.6.0 ± 24.7	29.5 ± 6.3	81.0 ± 5.8	134.5 ± 6.7	165.0 ± 8.2
OSI-9674 (n = 4)	1×10^{-4}	89.2 ± 17.5	33.8 ± 5.0	85.3 ± 6.4	127.3 ± 8.3	159.0 ± 8.0
	Control	100.0	21.75 ± 1.9	70.0 ± 5.0	125.4 ± 8.7	161.4 ± 7.8
	1×10^{-7}	88.2 ± 8.5	26.0 ± 4.9	74.7 ± 9.2	129.5 ± 6.0	168.0 ± 3.4
	1×10^{-6}	89.0 ± 9.8	27.0 ± 4.6	80.5 ± 11.0	137.0 ± 5.0	174.0 ± 5.0
	1×10^{-5}	65.8 ± 6.8	25.2 ± 5.7	76.6 ± 10.8	132.4 ± 7.5	174.6 ± 4.3
OSI-9675 (n = 4)	1×10^{-4}	42.0 ± 5.9	24.6 ± 6.0	70.3 ± 12.0	127.0 ± 6.6	171.7 ± 4.3
	Control	100.0	36.0 ± 9.8	88.4 ± 15.4	136.6 ± 11.5	179 6 ± 6.6
	1×10^{-7}	111.6 ± 6.7	36.0 ± 9.6	86.8 ± 15.0	136.3 ± 12	180.0 ± 7.0
	1×10^{-6}	110.0 ± 8.3	39.0 ± 8.7	90.0 ± 13.0	137.0 ± 9.2	180.7 ± 7.0
	1×10^{-5}	108.0 ± 11.2	44.0 ± 10.0	90.5 ± 10.3	139.0 ± 11	183.6 ± 7.4
OSI-9754 (n = 4)	1×10^{-4}	102.0 ± 13.7	43.0 ± 9.2	90.0 ± 15.3	137.5 ± 11	183.0 ± 7.2
	Control	100.0	33.0 ± 7.0	82.0 ± 12.4	124.5 ± 10.0	157 5 ± 7.2
	1×10^{-7}	80.6 ± 10.8	34.5 ± 6.0	80.8 ± 10.4	125.5 ± 5.9	162.0 ± 3.4
	1×10^{-6}	73.0 ± 4.8	32.8 ± 5.5	79.8 ± 8.3	130.3 ± 4.3	167.0 ± 3.6
	1×10^{-5}	67.6 ± 5.9	33.0 ± 5.5	79.8 ± 8.0	132.0 ± 2.9	167.0 ± 3.4
OSI-9757 (n = 4)	1×10^{-4}	50.9 ± 7.8	31.0 ± 3.2	77.3 ± 6.5	130.3 ± 4.0	172.0 ± 6.5
	Control	100.0	49.0 ± 6.6	109.5 ± 8.9	152.7 ± 6.0	180 7 ± 4.6
	1×10^{-7}	122.7 ± 11.2	50.0 ± 6.3	111.8 ± 7.8	158.5 ± 5.8	186.3 ± 7.2
	1×10^{-6}	122.9 ± 12.6	51.7 ± 5.8	112.0 ± 6.2	160.7 ± 4.0	188.0 ± 5.8
	1×10^{-5}	93.6 ± 10.8	50.0 ± 6.0	109.5 ± 5.2	158.7 ± 6.9	187.6 ± 6.1
OSI-9758 (n = 4)	1×10^{-4}	63.4 ± 11.0	54.7 ± 6.9	114.5 ± 6.5	160.5 ± 3.5	190.0 ± 2.6
	Control	100.0	32.0 ± 2.4	66.75 ± 5.9	97.0 ± 8.2	124.0 ± 5.9
	1×10^{-7}	76.0 ± 9.6	32.0 ± 2.2	64.0 ± 5.3	92.6 ± 6.7	121.0 ± 5.8
	1×10^{-6}	58.4 ± 8.3	31.0 ± 3.0	63.4 ± 6.2	89.6 ± 7.3	118.2 ± 4.8
Cerebrocrast	1×10^{-5}	43.47 ± 5.8	28.4 ± 2.6	59.0 ± 4.4	88.0 ± 4.9	118.7 ± 4.6
OSI-1212 (n = 4)	1×10^{-4}	26.4 ± 2.8	29.0 ± 2.6	55.6 ± 2.9	83.6 ± 3.6	112.7 ± 6.4
	Control	100.0	21.25 ± 3.0	59.2 ± 7.7	109.0 ± 6.0	147.2 ± 6.0
	1×10^{-7}	79.5 ± 2.0	21.6 ± 3.6	59.2 ± 6.9	107.2 ± 5.3	145.0 ± 3.8
	1×10^{-6}	78.2 ± 11.6	20.0 ± 2.8	57.9 ± 5.9	99.0 ± 3.2	136.2 ± 4.3
OSI-3802 (n = 5)	1×10^{-5}	52.7 ± 11.6	18.4 ± 1.9	51.4 ± 5.5	96.0 ± 4.2	134.75 ± 3.9
	1×10^{-4}	32.8 ± 5.3	16.2 ± 1.7	41.75 ± 5.6	81.6 ± 5.6**	118.75 ± 7.2**
	Control	100.0	42.3 ± 3.4	114.8 ± 3.9	171.0 ± 9.7	195.7 ± 10.9
	1×10^{-7}	94.0 ± 11.0	42.3 ± 1.7	1119.0 ± 3.9	176.5 ± 10	202.7 ± 10.0
	1×10^{-6}	80.0 ± 13.3	52.3 ± 5.7	129.0 ± 6.3	183.5 ± 11	213.0 ± 13.4
	1×10^{-5}	67.7 ± 12.7	50.7 ± 7.0	130.7 ± 5.4	188.0 ± 11	217.0 ± 13.9
OSI-9879 (n = 4)	1×10^{-4}	67.3 ± 11.9	47.3 ± 6.3	126.7 ± 4.4	183.7 ± 11	211.0 ± 13.5

Table (continued)

1	2	3	4	5	6	7
	Control	100.0	38.0 ± 7.4	110.0 ± 11.4	174.6 ± 10	207.5 ± 9.6
	1×10^{-7}	103.0 ± 9.8	41.0 ± 7.2	116.6 ± 9.5	185.6 ± 6.6	220.0 ± 7.7
	1×10^{-6}	93.2 ± 13.5	43.6 ± 8.0	123.0 ± 10.0	196.4 ± 8.5	229.5 ± 9.7
	1×10^{-5}	92.5 ± 12.6	42.6 ± 8.0	124.6 ± 8.4	202.0 ± 8.9	232.5 ± 9.4
OSI-9761 (n = 4)	1×10^{-4}	92.0 ± 11.7	47.0 ± 6.6	133.6 ± 12.0	209.0 ± 6.6*	242.0 ± 8.9*
	Control	100.0	43.0 ± 5.6	100.0 ± 7.0	146.8 ± 6.9	174 0 ± 6.0
	1×10^{-7}	104.0 ± 12.0	43.5 ± 5.7	102.0 ± 7.0	150.0 ± 6.0	179.0 ± 2.5
	1×10^{-6}	95.6 ± 15.0	42.0 ± 6.0	101.0 ± 7.2	148.7 ± 7.0	177.0 ± 7.0
	1×10^{-5}	81.0 ± 12.8	42.0 ± 7.3	103.5 ± 6.5	150.0 ± 7.7	177.0 ± 7.3
OSI-9843 (n = 4)	1×10^{-4}	67.8 ± 9.3	45.0 ± 6.0	104.0 ± 8.0	153.0 ± 9.0	181.8 ± 8.4
OSI-4161 (n = 4)	Control	100.0	39.0 ± 8.6	96.0 ± 12.0	161.0 ± 12	198.0 ± 4.3
	1×10^{-7}	78.85 ± 12.6	36.0 ± 8.0	90.6 ± 12.2	153.0 ± 6.9	190.0 ± 5.8
	1×10^{-6}	73.5 ± 14.3	35.0 ± 7.9	93.7 ± 11.8	161.0 ± 5.7	202.0 ± 7.1
	1×10^{-5}	90.3 ± 16.0	39.5 ± 4.4	111.6 ± 3.8	177.0 ± 2.1	217.0 ± 8.0
	1×10^{-4}	92.8 ± 16.0	39.5 ± 6.9	112.6 ± 6.3	178.0 ± 4.9	220.0 ± 7.5
OSI-9963 (n = 4)	Control	100.0	37.8 ± 2.2	86.7 ± 5.2	132.7 ± 7.8	169.0 ± 7.0
	1×10^{-7}	101.0 ± 7.3	40.0 ± 1.5	92.5 ± 5.7	138.0 ± 13.0	171.5 ± 8.6
	1×10^{-6}	98.5 ± 10.0	38.5 ± 1.5	91.4 ± 6.3	140.5 ± 10.5	172.0 ± 8.0
	1×10^{-5}	103.0 ± 10.2	44.7 ± 4.8	103.2 ± 3.2*	149.8 ± 7.7	175.5 ± 11.2
	1×10^{-4}	97.8 ± 8.2	47.0 ± 1.0**	111.3 ± 6.4*	156.5 ± 12.5	184.0 ± 13.0
OSI-9968 (n = 5)	Control	100.0	30.0 ± 2.2	84.0 ± 10.3	136.4 ± 12.8	163.0 ± 12.7
	1×10^{-7}	89.5 ±7.7	35.0 ± 6.7	91.0 ± 12.4	136.6 ± 12.3	166.7 ± 12.3
	1×10^{-6}	83.7 ± 9.5	36.0 ± 7.2	96.0 ± 14.4	149.6 ± 12.2	180.0 ± 9.4
	1×10^{-5}	81.0 ± 9.7	37.5 ± 6.0	103.0 ± 11.3	161.2 ± 12.5	188.0 ± 11.5
	1×10^{-4}	73.0 ± 10.6	38.2 ± 8.3	101.0 ± 10.8	158.7 ± 12.9	189.6 ± 11.3
OSI-9719 (n = 6)	Control	100.0	37.0 ± 5.7	107.4 ± 10.0	176.0 ± 8.4	206.0 ± 10.4
	1×10^{-7}	108.0 ± 3.7	40.0 ± 7.0	112.7 ± 10.6	188.5 ± 7.0	221.6 ± 9.4
	1×10^{-6}	120.0 ± 6.0	41.4 ± 7.7	118.6 ± 9.8	196.0 ± 6.8	229.0 ± 7.0
	1×10^{-5}	134.0 ± 8.8	46.0 ± 6.3	133.0 ± 5.9	205.8 ± 5.7	238.5 ± 5.9*
	1×10^{-4}	190.8 ± 53.7	55.5 ± 5.0*	146.9 ± 5.7**	222.0 ± 5.2***	256.0 ± 4.0***

Data are expressed as means ± SE. Stimulation rate is 1 Hz; P is contraction force at a given concentration; P_0 is initial contraction (100%); APD_{10}, APD_{25}, APD_{50}, APD_{90} are action potential duration at 10, 25, 50 and 90% repolarization; n is a number of preparations (papillary muscles); control is physiological solution, containing less than 1 μM DMSO; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs pre-drug value. #EJMC 2011, chemical structures of pointed compounds, are in (20); see references.

RESULTS AND DISCUSSION

The analysis of the chemical structure of 1,4-dihydropyridine derivatives and their effects on the papillary muscle contraction force and the action potential duration has shown that the majority of the tested compounds have negative inotropic properties, which depend on the nature of the substituents in phenyl and dihydropyridine rings, spatial isomerization and are inversely proportional to the used concentrations. The maximum contraction force expression was registered at the doses of 10^{-4} mol/L. The synchronous registration of the action potential (AP) duration has revealed that the

AP duration variations do not always coincide with the variability of the isometric contraction.

Amlodipine (OSI-9787), used in the concentration-dependent manner, significantly decreased the contraction force of guinea pig papillary muscles and it reached 60% compared to the baseline at a concentration of 10^{-4} mol/L, while APD has varied insignificantly toward narrowing, except the early depolarization (APD_{10}), which decreased by 5.25 ms, 13.6% ($p < 0.05$, versus the initial value).

By substitution of amine group hydrogen for benzensulphonate, the compound (OSI-9529, Norvasc) was synthesized, which, according to its effectiveness, was very similar to amlodipine: the contraction force decreased by 64% (10^{-4} mol/l) and the AP duration narrowed negligibly. Similar data were obtained when hydrogen was substituted by acetyl- compound (OSI-9528). Under its influence, the contraction force of papillary muscles decreased by 63.4%, APD did not change, while the other substituent, 2,5-dihydrobenzoate (OSI-9587), resulted in 1.5 times less impact on the contraction force and no impact on the AP duration.

The nature of the substituents in the phenyl ring has significant influence on the efficiency of the presented compounds. This confirms the results obtained by the study of the derivative OSI-9614, in the structure of which a chlorine atom in phenyl rings was substituted by diflormethoxy- radical (compared with the structure of OSI-9529). In this case, the negative inotropic effect was even more pronounced, and the APD significantly decreased at 10 and 25% repolarization (35.7 and 31.4%, respectively). Substitution of the amine group in the amlodipine molecule by ethylene glycol (derivatives OSI-9730, OSI-9566, OSI-9586) or much more complex group – 2,2-dimethyl-[1,3] dioxolan-4-ylmethoxymethyl- (OSI-9674) and additional incorporation of a chlorine atom into the phenyl ring (OSI-9599, OSI-9600) did not show any substantial differences in respect of isometric contraction and AP. Under the influence of these compounds, the contraction force of the papillary muscles decreased by 49–53% and the AP duration fluctuated insignificantly.

Other group compounds, according to their chemical structure, are similar to this one of amlodipine characterized by spatial isomerism. Thus, an isomer of the right rotation (OSI-9674) slightly increased the contraction force only at the low concentrations (10^{-7}–10^{-6} mol/L), however, the contraction force at the highest concentration (10^{-5}–10^{-4} mol/L) slightly decreased (~10%), while the AP duration lengthened by 15.6, 17.3 and 11.8%, respectively, at 25, 50 and 90% repolarization. At the same time, the isomer of the left rotation (OSI-9675) decreased the isometric contraction by 58%, however, its action on the APD was not registered. The mix of these compounds (racemate OSI-9597) conditioned the significant negative inotropic properties, that is, by its action the contraction force of papillary muscles decreased about 70% at a concentration of 10^{-4} mol/L although the AP duration did not change at all repolarization levels. The analogous results were received with the cerebrocrast-amlodipine's hybrid. The hybrid of the right rotation (OSI-9758) at the low concentrations increased the contraction force (~22%), while at the higher doses (10^{-5}–10^{-4} mol/L) decreased it by ~37%. The action potential duration varied negligibly. The isomer of the left rotation (OSI-9757) reduced the isometric contraction up to 49% at a concentration of 10^{-4} mol/L, the APD varied negligibly as well. Meanwhile, the racemate (OSI-9754), unlike the previously studied compounds, did not show any marked effect neither on the isometric contraction nor on the AP duration.

The next group of the presented 1,4-dihydropyride derivatives according to their chemical structure was different to the above investigated compounds mainly by the nature of substituents in dihydropyridine and phenyl rings. So, the compound OSI-1212, known as cerebrocast (CAS [118790-71-9]), has a diflormethoxy- group in the second position of the phenyl ring and a (propoxy)ethoxycarbony- group in the 3rd and 5th positions of the dihydropyride ring. Such transformation of the chemical structure strengthened the negative inotropic action compared to that of amlodipine, while their (amlodipine and OSI-1212) effects on the AP duration did not differ substantially from one another. Meanwhile, the compound OSI-3802, which chemical structure differs from OSI-1212 by that propoxy- radical, was substituted by isopropoxy- one, significantly narrowed the AP duration (29.5, 25 and 19.3%, respectively, at 25, 50 and 90% repolarization) in parallel reducing the contraction force by 67.2%.

Other variations in the chemical structure, which represent the compounds OSI-9789, OSI-9879, OSI-9761 and OSI-9843, resulted in a weaker negative inotropic effect and AP duration. So, the compound

OSI-9761 in wide concentration ranges did not reduce the contraction force of papillary muscles, however, significantly ($p < 0.05$) prolonged the action potential duration (23.6, 34.4, 35 ms at 25, 50 and 90% repolarization).

Compounds, having in their structure $–NO_2$ substituent in position 5 of the 1,4-dihydropyridine ring and in positions 2 and 4 of the phenyl ring triflormethyl- (CF_3) or diflormethoxy- (OCF_2) groups, possess both positive and negative inotropic properties and have shown a significant impact on the AP duration. So, the isometric contraction of isolated guinea pig papillary muscles under the influence of OSI-9961 increased on the average by 11.5% irrespective of the concentration used. However, a compound which has not 6-methyl- radical (OSI-4146) shows a negative inotropic effect and in parallel increases the AP duration, more signally at 90% repolarization (22.0 vs 7.9 ms OSI-4146 and OSI-9961, respectively). A similar effect was recorded in the OSI-9962 and OSI-9963 groups: the absence of methyl radical in the 1,4-dihydropyridine ring conditioned the negative inotropic properties and statistically significant lengthening of AP duration, more pronounced at early depolarization (APD_{10} and APD_{25}). Substitution of the 2-flormethoxy- for the 2-triflormethyl- (OSI-4161 and OSI-9963, respectively) did not significantly influenced the isometric contraction and AP duration. Meanwhile, analogical substitution in the case of OSI-9962 and OSI-9961 increased the positive inotropic effect only. Another compounds of a similar structure (OSI-9968 and OSI-9719) studied which differ from each other by a nature of substituents and their position in the phenyl ring (4-triflormethyl- and 2-triflormethoxy-) in respect of APD showed a similar effect. The efficiency analysis of compounds OSI-4146 and OSI-9719 showed that the 2-propoxyethyl- substituent determines not only further lengthening of APD but also strengthens the positive inotropic effect. So, the AP duration in the OSI-9719 group at the doses of 10^{-4} mol/L increased by 18.5, 39.5, 46 and 50 ms, respectively at 10, 25, 50 and 90% repolarization, and this is consistent with the augmentation of isometric contraction of the papillary muscle. The variations of APD in OSI-9962 as well as in OSI-2456 groups were insignificant although both compounds possess the positive inotropic properties. They are known respectively as Bay K 8644 (CAS [71145-03-4]) and CGP 28392 (CAS [89289-93-0]), respectively.

We have concluded that the majority of the 25 investigated 1,4-dihydropyridine derivatives possessed the negative inotropic properties and had the negligible impact on the action potential duration. The relationship between the contraction force of papillary muscles and the action potential duration was not established. However, the compound OSI-9719 (2-propoxyethyl-4-(difloemethoxyphenyl)-2-methyl-5-nitro-1,4-dihydropyridine-3-carboxylate) significantly ($p < 0.05$) prolonged the duration of AP and simultaneously increased the force of contraction and according to the positive inotropic effect is equivalent to the Ca^{2+} channel agonist Bay K 8644. Efficiency of the investigated compounds depends on the nature of the substituents in the phenyl and 1,4-dihydropyridine rings as well as on their spatial isomerization and is concentration-dependent.

Screening of the investigated 1,4-dihydropyridine derivatives possessing the cardiotonic properties discovered a good predictive potential of the applied approach: the contraction force of papillary muscles appeared to be a more informative indicator compared to that of the action potential duration.

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KARDIOMIOCITŲ SUSITRAUKIMO JĖGOS IR VEIKIMO POTENCIALO TRUKMĖS TYRIMAS: KURIS METODAS INFORMATYVESNIS EKSPERIMENTO SĄLYGOMIS TIRIANT 1,4-DIHIDROPIRIDINO JUNGINIŲ EFEKTYVUMĄ?

Santrauka

Tikslas. Svarbus vaidmuo naujai sintezuotų cheminių junginių aktyvumui įvertinti tenka pasirenkant eksperimentinio tyrimo metodą.

Eksperimentinių tyrimų tikslas – nustatyti 25 tirtų 1,4-dihidropiridino junginių cheminės struktūros ir jų poveikio raumenų susitraukimo jėgai ir veikimo potencialo trukmei (VP) tarpusavio ryšį.

Tyrimo metodai. VP trukmė matuota standartiniais mikroelektrodais, pagamintais iš boro silikatinio stiklo kapiliarų, pripildytų 2,5 M KCI ir prijungtų prie aukštos įeinamosios varžos stiprinimo sistemos. Taikytas 1,0 Hz stimuliacijos dažnis. Susitraukimo jėga registruota specializuotu jėgos davikliu. VP trukmės ir susitraukimo jėgos signalai apdoroti A/D keitikliu ir registruoti naudojant specializuotą kompiuterinę programą.

Rezultatai. Eksperimentų duomenys rodo, kad dauguma 1,4-dihidropiridino junginių pasižymi neigiamu inotropiniu veikimu ir turi nereikšmingą įtaką veikimo potencialo trukmei.

1,4-dihidropiridino junginys OSI 9719 (*2-propoksietil-4-diflormetoksi-2-metil-5-nitro-1,4-dihidropiridino-3-karboksilatas*) padidino veikimo potencialo trukmę, kartu padidindamas kardiomiocitų susitraukimo jėgą ($p < 0,05$).

Tirtų junginių efektyvumas priklauso nuo cheminių grupių (diflormetoksi-, etilenglikolio-, izopropoksi-, chloro atomo, amino, (propoksi)etoksikarbonyl-) prigimties, jų padėties fenilo ir 1,4-dihidropiridino žieduose bei tiriamųjų junginių koncentracijų.

Išvados. Jūros kiaulyčių papiliarinių raumenų susitraukimo jėgos vertinimas yra informatyvesnis metodas, palyginti su veikimo potencialo trukmės vertinimu vykdant 1,4-dihidropiridino junginių efektyvumo eksperimentinius tyrimus.

Raktažodžiai: 1,4-dihidropiridino dariniai, inotropinis poveikis, veikimo potencialo trukmė, jūrų kiaulyčių papiliariniai raumenys