



Contractility of Internal Thoracic Artery in Patients Undergoing Coronary Artery Bypass Grafting

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Abstract: The objective of the study was to estimate the contractility characteristics and electromechanical coupling of human internal thoracic artery (ITA) by mechanography and double sucrose gap technique to compare the effect of nitroglycerin with other pharmacological agents. The study of mechanical tension (mechanography) of the ITA segments has been performed in 14 patients undergone coronary artery bypass grafting (CABG). After provisional straining by 500 mg preload, the mechanical tension was registered in real time on two-coordinate plotter of mechanoelectrical converter. For simultaneous registration of membrane potential (MP) and mechanical tension (MT) during cultivation in pharmaceuticals, the method of double sucrose gap technique was used. The maximal MT of ITA segments was achieved under the exposure to potassium chloride. Paradoxical ITA contractility reaction was observed during the application of niflumic acid. Phenylephrine had no statistically significant effect on the ITA MT even at high concentrations. Epinephrine caused a pronounced contractile response of the ITA segments, which was similar to potassium chloride. Hyperpotassium-induced MT of the distal ITA segments was 3.5 times higher than this of the proximal ones. There were no statistically significant differences in the contractile response of the ITA segments studied on the same day as a surgery or the next day. A direct correlation between nitroglycerin vasodilating effect and its dose has been found. There was no restoration of the MT to the baseline values after the nitroglycerin-induced maximal relaxation followed by a wash with Krebs solution. There was an exponential relationship between the concentrations of nitroglycerin required to achieve the maximal relaxation and the initial MP. Conclusion: The maximum potassium-induced MT of the distal ITA segments was significantly higher than this of the proximal ones. There was no effect of phenylephrine on the ITA MT. Epinephrine and niflumic acid cause pronounced ITA contractile response. Nitroglycerin has a marked relaxing influence on ITA and a maximal effect at a low level of initial MP. Therefore, if MP increases, high doses of nitroglycerin are required to achieve maximum vasodilation.

Keywords: Internal Thoracic Artery, Contractility, Electromechanical Coupling, Mechanography

1. Introduction

The occurrence of the autoarterial graft spasms in coronary artery bypass grafting is associated with increased morbidity and mortality after ischemic episodes and perioperative myocardial infarction [1], [2]. Mechanisms regulating contractile function of internal thoracic artery (ITA) and its causes are not fully understood. Some authors suggested that sympathectomy (especially after skeletonization), inflammatory response to a release of contractile mediators [3], etc. are actively involved in this process. Inotropes

increase arterial contractile response by increasing myocardial oxygen consumption, whereas artery spasms reduce oxygen delivery [4].

One of the basic mechanisms underlying the development of ITA spasm is a mismatch of blood volume flowing through narrowed autoarterial bypass graft to submaximal consumption of revascularized myocardium, stationed under functional overload in the immediate postoperative period. P. A. Spence, et al. [5] suggested that supramaximal arterial blood flow through narrowed conduits would decrease the distal intraluminal pressure, thereby reducing ITA smooth

muscle pressure load and rendering the arterial graft vulnerable to spasm. In vitro studies of porcine ITAs and gastroepiploic arteries demonstrated that narrowed conduits develop large transconduit pressure gradient under increased myocardial blood consumption. Under these conditions, autoarterial grafts are very sensitive to vasoconstrictor influences [6]. Previously, heterogeneity of vasoconstrictive potential in ITA has been reported: distal segments were significantly more sensitive to serotonin than proximal ones [7]. This effect was greater in patients with arterial hypertension than in normotensive patients. Therefore, hypertensive patients are more prone to perioperative ITA spasms.

Topical vasodilator agents, in particular papaverine, are widely used to reduce ITA spasms during and after harvesting [8], [9], [10]. Nonetheless, several authors reported their inefficiency, or at least no significant difference in relaxing effect compared to other agents [11], [12]. Postoperative infusion of papaverine is not routinely, because high concentrations, required to produce vasodilator effect, are associated with marked hypotensive consequences. Therefore, some researchers propose to use nitrates as a promising drug for the prevention of ITA spasm [13], [14], [15], [16], [17], [18]. However, C. Huraux *et al.* [19] reported that many coronary artery disease patients undergoing CABG had nitrate tolerance. Therefore, their application as vasodilators may be ineffective during and immediately after surgery. The authors suggested to use isradipine, which reduced ITA spasms by 100% at a mean therapeutic concentration, whereas nitroglycerin reduced spasms only by 50%. Recently, much attention has been focused on calcium antagonists which may reduce ITA contractility and are regarded as effective antispasmodics in the intraoperative and immediate postoperative periods [3], [13], [19], [20], [21].

All the above-mentioned studies were limited by the use of isolated artery segments; they did not consider muscle fiber architecture. Moreover, the characteristics of contractility of different segments and electromechanical coupling have not been studied, the effect of skeletonization or pedicled harvesting was also not taken into account. Finally, none of the studies were performed on human ITAs.

The objective of the study was to estimate the contractility characteristics and electromechanical coupling of human internal thoracic artery (ITA) by mechanography and double sucrose gap technique to compare the effect of nitroglycerin with other pharmacological agents.

2. Materials and Methods

The study was approved by the Local Ethics Committee. All patients provided written informed consent. The objects of the biophysical study were 20 isolated smooth muscle (SM) strips of human ITAs which were harvested from 14 patients undergoing off-pump coronary artery bypass grafting (CABG) using one or two ITAs. The arteries were cultivated in Krebs solution until the experiment, which was performed on the same day as a surgery or on the next day. The

experimental arterial segments were cleared from adhering fat and connective tissue (adventitia) before the experiment. Each arterial segment was cut into isolated rings (3-5 mm in length). The endothelium was removed by mechanical debridement, by gently rotating the vessel segment about a wooden dowel inserted through the lumen of the vessel ring. The removal of the endothelium was verified by the light supravital microscopy. The deendothelized arteries were cut 0.5 mm in width and 14-16 mm in length according to the direction of main muscular fiber layer (the mean circumferential angle 65° to the longitudinal axis of the vessel or 25° to the transversal one) (Figure 1, A). The tissue samples were incubated in the Krebs solution containing (mM): 120.4 NaCl, 5.9 KCl, 2.5 CaCl_2 , 1.2 MgCl_2 , 5.5 glucose and 15 $\text{C}_4\text{H}_{11}\text{O}_3\text{N}$ (tris(hydroxymethyl)-aminomethane), pH 7.4 at a temperature of 20-25°C.

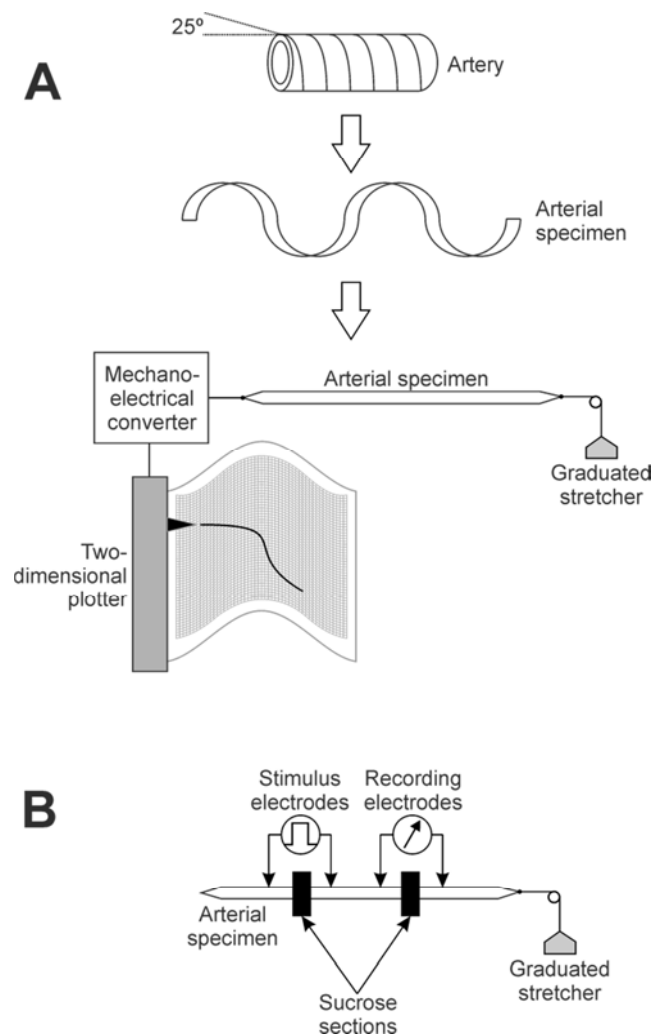


Figure 1. The scheme of experiment: A – the scheme of ITA specimen preparation and the mechanographical stand; B – the scheme of a modified double sucrose gap chamber.

For isometric muscular tension recordings the arterial sample was mounted on the mechanoelectrical stand after the pre-load tension of 500 mg. The sample was pushed in the cuvette and continuously perfused with equilibrated Krebs

solution at a rate of 1 ml/min via the cuvette. The temperature was kept constant at 36.8-37°C. Then the samples underwent a normalizing procedure by washing with the Krebs solution for 45-50 min. After the normalizing procedure at least two active contractions were then induced by hyperpotassium depolarization (KCl, 30 mM). After a steady level of potassium-induced contractions was established, the samples were washed and one hyperosmotic contraction was generated using the modified Krebs solution, containing 150 μ M sucrose followed by a 30-min wash.

The samples were exposed to the studied pharmaceuticals (a 15-40-min pretreatment depending on the drugs' characteristics). The amplitude of the contractile responses of smooth muscle segments was calculated in mN or as a percentage of the amplitude of the control hyperosmotic contraction.

The double sucrose gap technique was used for SM electromechanical coupling registration [22]. The essence of the technique is the use of two chambers containing sucrose solution to isolate a tissue, which is immersed in a physiological solution. The two ends of the tissue are depolarized by a solution rich in potassium ions. The membrane potential (MP) differences between the node, or the test chamber, and one of the potassium-rich chambers can be measured; while the potential in the node can be modified by the current degenerated between the other potassium-rich chamber and the node. The modified double sucrose gap can be used for simultaneous recording of MP and MT as well (Figure 1, B). For this purpose, the muscular strip was placed

in the double sucrose chamber with its ends fixed in the stretcher of mechano-electrical converter. Then the SM specimen was stretched by 250 mg preload to a length close to the original. The perfusion rate of the Krebs solution and the test pharmaceuticals through the test chamber was 1 ml/min. Hyperpotassium (30 mM KCl) and nitroglycerin solutions were prepared on the base of the Krebs solution. Rectangular pulses with amplitude of 0.1-0.7 μ A and duration of 3-5 seconds were used as a stimulus polarizing current. The MP was registered using non-polarizable agar-agar bridged electrodes with the resistance less than 2 K Ω .

The variables were presented as median and quartile range. All statistical calculations were done with the StatPlus Pro software package (AnalystSoft, Canada) using the Mann-Whitney, Friedman, Newman-Keuls, Dunnett and Spearman tests. $P < 0.05$ was considered statistically significant.

3. Results

The typical pattern of the of ITA contractility response curves consists of several phases. The contraction begins 2-3 minutes after the vasoconstrictive pharmaceutical application; a continuous increase of the contraction lasts 20-30 minutes until the plateau is reached [23]. The relaxation occurs 2-3 minutes after washing in the Krebs solution or the application of nitroglycerin and lasts 15-20 minutes until the full restoration of SM tone (Figure 2). Table 1 shows the values of MT of the ITA segments exposed to the solutions of various contractile agents.

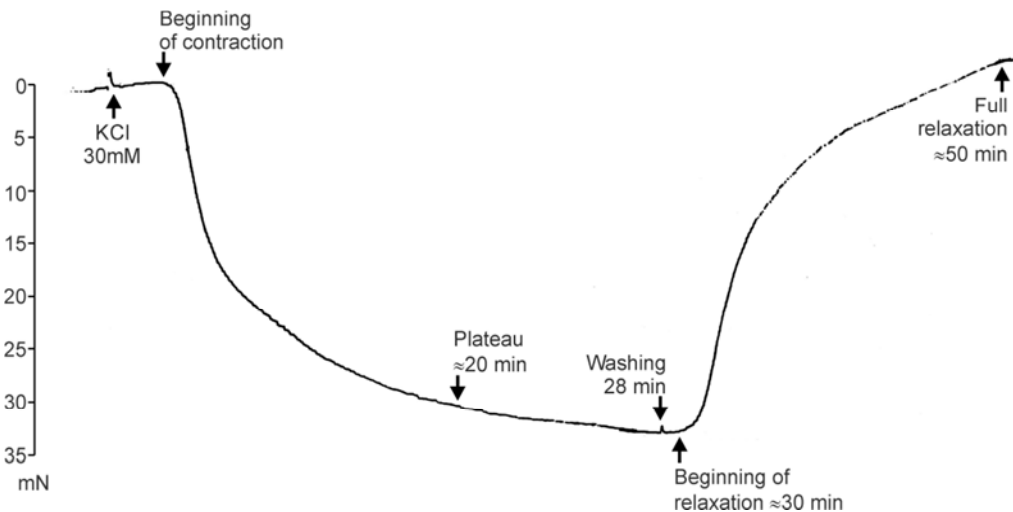


Figure 2. Typical curve of ITA contractility response (mechanogram) under contractility pharmaceutical application (30 mM KCl solution in this case) and subsequent washing in the Krebs solution.

Table 1. The values of ITA segments MT during exposure in solutions of various contractile agents.

Solution	Maximal MT, mN	P in comparison with Krebs solution*
Krebs solution (1)	0 (-1.3; 3.3)	-
Phenylephrine 10^{-7} (2)	-1.6 (-1.9; -0.3)	0.815
Phenylephrine 10^{-6} (3)	-0.4 (-2.2; 1.8)	0.545
Phenylephrine 10^{-5} (4)	4.7 (3.1; 12.7)	0.063
Niflumic acid 10^{-7} (5)	24.3 (20.2; 29)	0.021
Epinephrine 10^{-7} (6)	25 (20; 31.5)	0.013
KCl 30 mM (2)	32.3 (25.1; 40.3)	0.005

Comment. All data are presented as median, lower and upper quartile. * - by Dunnett test; $P = 0.004$ by Friedman test.

The maximal MT of ITA segments was achieved under the exposure to potassium chloride. Interestingly, paradoxical ITA contractility reaction was observed during the application of niflumic acid. It was similar to epinephrine in magnitude (Table 1). Simultaneous application of potassium chloride and niflumic acid (Figure 3) revealed their

potentiating action on hyperpotassium-induced ITA contraction. The magnitude of niflumic-potentiated contraction component corresponded to the magnitude of isolated niflumic-induced contractions or sequential use of potassium chloride and niflumic acid.

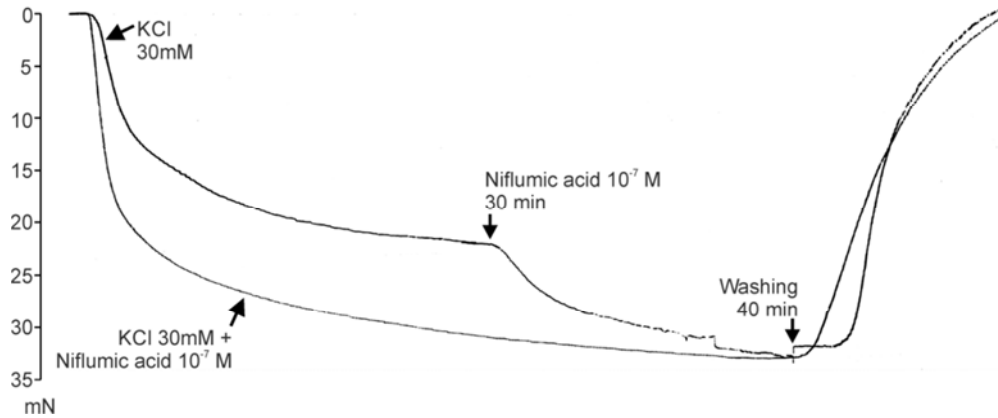


Figure 3. The MT curves (mechanograms) demonstrating additive effect of potassium chloride and niflumic acid simultaneous or sequential application.

Phenylephrine had statistically significant effect on ITA MT only at high concentrations. Epinephrine, on the contrary, caused a pronounced contractile response of ITA segments, which among other drugs was close to that under the influence of potassium chloride.

The maximal MT and the rate of its reaching had

statistically significant dependence on the segment of ITA (Table 2): hyperpotassium-induced MT of the distal ITA segments was 3.5-fold higher compared to the proximal ones. There were no statistically significant differences in the contractile response of ITA segments studied on the same day as a surgery or the next day.

Table 2. The maximal MT and rate of its reaching depending on the segment of ITA and the day after harvesting.

Parameter	Maximal MT, mN	Rate of maximal MT reaching, s
KCl, proximal segment (1)	21.1 (16.5; 23.4)	1090 (1010; 1140)
KCl, distal segment (2)	43.8 (36.6; 48.2)	870 (820; 950)
Niflumic acid, proximal segment (3)	14.9 (12.3; 16.9)	1350 (1280; 1410)
Niflumic acid, distal segment (4)	26.4 (22.1; 29.5)	980 (910; 1040)
KCl, the 1 st day (5)	33.2 (26.7; 37.2)	1120 (1070; 1160)
KCl, the 2 nd day (6)	29.1 (25.5; 34.9)	1110 (1050; 1170)
Niflumic acid, the 1 st day (7)	18.3 (15.2; 22.1)	1080 (990; 1120)
Niflumic acid, the 2 nd day (8)	16.1 (13.7; 19.3)	950 (1020; 1060)
P (1-2)*	0.022	0.018
P (3-4)*	0.036	0.020
P (5-6)*	0.113	0.615
P (7-8)*	0.377	0.243

Comment. All data are presented as median, lower and upper quartile. * - by Mann-Whitney test.

Table 3 demonstrates electromechanical coupling data during nitroglycerin test after hyperpotassium contraction using double sucrose gap technique. A direct correlation between nitroglycerin vasodilating effect and its dose was shown. It reduces hyperpotassium-induced MT at concentrations of 10^{-8} - 10^{-7} M, but not to the initial values. With the concentration increased to $5 \cdot 10^{-7}$ M, a marked decrease of MT below the baseline was observed. The complete relaxation occurred at the nitroglycerin concentration of $5 \cdot 10^{-6}$ M. Importantly, there was no restoration of the MT to the baseline values after the nitroglycerin-induced maximal relaxation followed by a wash with Krebs solution.

Table 3. The maximal MT and MP during nitroglycerin test after hyperpotassium contraction using double sucrose gap technique.

Parameter	Maximal MT, mN	Maximal MP, mV
Krebs solution (1)	0 (-1.1; 2.3)	0 (-0.2; 0.2)
KCl 30 mM (2)	21.7 (17.2; 24.1)	+7.6 (+5.5; +8.2)
Nitroglycerine 10^{-8} (3)	17.7 (14.5; 20.8)	+7.4 (+5.2; +7.9)
Nitroglycerine $5 \cdot 10^{-8}$ (4)	12.6 (9.9; 14.6)	+7.1 (+4.8; +7.6)
Nitroglycerine 10^{-7} (5)	5 (1.8; 7.7)	+6 (+4.4; +7.1)
Nitroglycerine $5 \cdot 10^{-7}$ (6)	-3.8 (-7.5; -0.2)	+7.1 (+4.2; +7.8)
Nitroglycerine 10^{-6} (7)	-10.5 (-16.6; -6.4)	+6.5 (+6.1; +6.9)
Nitroglycerine $5 \cdot 10^{-6}$ (8)	-48.9 (-51.2; -27.9)	+6.5 (+6.2; +6.7)
Krebs solution (9)	-23.6 (-26.8; -12.7)	0 (-0.5; +0.5)
P*	0.001	0.003
P (1-2)**	0.007	0.012

Parameter	Maximal MT, mN	Maximal MP, mV
P (2-3)**	0.213	0.745
P (2-4)**	0.083	0.844
P (2-5)**	0.041	0.843
P (2-6)**	0.014	0.434
P (2-7)**	0.008	0.458
P (2-8)**	0.001	0.252
P (8-9)**	0.051	0.009
P (1-9)**	0.020	0.816

Comment. All data are presented as median, lower and upper quartile. * - by Friedman test; ** - by Newman-Keuls test.

As expected, nitroglycerin does not change the MP of ITA SM. However, there was an exponential relationship between the concentrations of nitroglycerin required to achieve maximal relaxation and the initial MP before its application (Figure 4). Nitroglycerin has a maximal effect at a low level of initial MP. However, if MP increases, high doses of nitroglycerin are required to achieve maximum vasodilation.

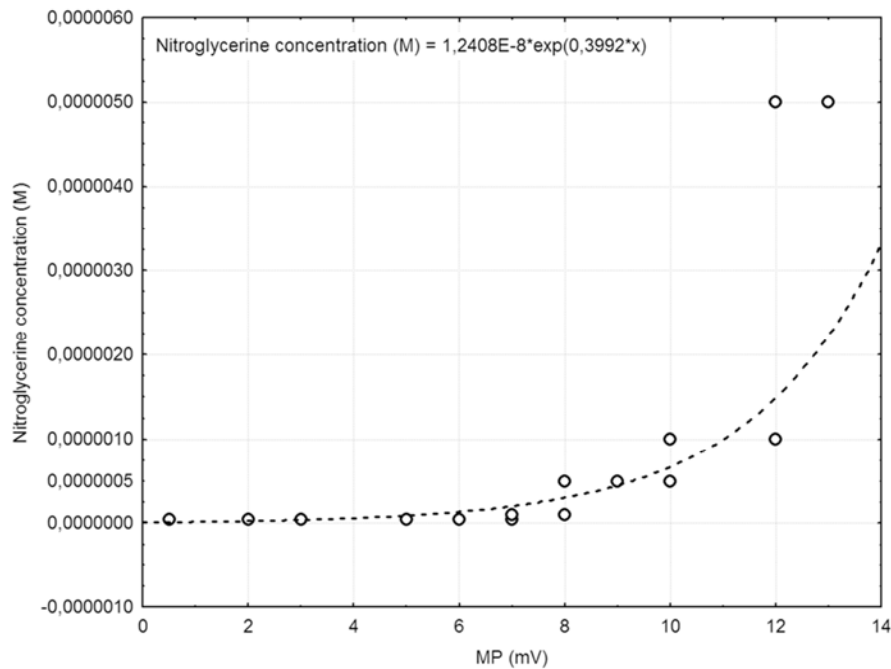


Figure 4. Exponential relationship between the concentrations of nitroglycerin required to achieve maximal relaxation and the initial MP before its application (Spearman $R=0.946$, $P<0.0056$).

4. Discussion

The study of ITA contractile properties in the short-term postoperative period and the effects of nitroglycerin was performed in 14 patients undergoing off-pump CABG. The objects of the study were isolated deendothelized smooth muscle segments. Using the technique of double sucrose bridge the amplitude of contraction and the membrane potential were simultaneously recorded under the application of various contractile agents and nitroglycerine application.

Mechanographic data demonstrated some characteristics of the contractile response of ITA. In particular, phenylephrine does not significantly affect the SM tone, while niflumic acid paradoxically increased it. The most significant MT comparable to hyperpotassium one was achieved under the application of norepinephrine. This fact indirectly testifies that epinephrine, which is widely used as an inotropic agent in the postoperative period, is not recommended to be used, since it provokes ITA spasm.

In case of sequential exposure of ITA to potassium chloride and niflumic acid we found out that that the mechanisms of potassium-induced and contraction niflumic-

induced contraction are different. Moreover, an additive effect may develop when they are combined. This allows to conclude that the potassium and chlorine membrane transport in ITA seems to be disconnected in contrast to SM of intestines. The contraction of ITA SM may also be associated with cyclooxygenase block induced by niflumic acid instead of chlorine channel block mechanisms. Possibly, this phenomenon is associated with the features of the redistribution of chloride ions in case of blocking chlorine currents in ITA SM cells, that for some reason causes an increase in the intracellular calcium ion concentration. Further studies are required to investigate this process.

The obtained findings suggested that the distal segments of the ITA showed a more pronounced maximum rate of MT achievement than the proximal ones. Therefore, in case of enough graft length, it is recommended to avoid the use of the distal segment and cut off. This conclusion is consistent with the results of other studies [24], [25], [26]. The maximum contractile activity of ITA was found on the second day after the surgery, which corresponds to the clinical data of the highest severity of ITA spasms and is associated with ischemic episodes during this period. It means that skeletonized ITAs are subjected to spasms not

only immediately after harvesting but also during a more prolonged period, and therefore requires continuous prevention.

A direct correlation between vasodilating effect of nitroglycerin and its dose has been found. It reduced the potassium-induced MT at concentrations of 10^{-8} – 10^{-7} M, but not to the initial values. Increased concentration up to $5 \cdot 10^{-7}$ M provoked a marked tone reduction below baseline values. The complete relaxation of ITA occurred at a nitroglycerin concentration of $5 \cdot 10^{-6}$ M.

It is important to note that the contractile reserve of ITA segments was significantly lower than the relaxation reserve induced by nitroglycerin. It means that in the first day after surgery the ITA has a high basal tone close to its maximal values. Another noteworthy phenomenon is that the arterial tone does not return to the baseline values after the maximal nitroglycerine-induced relaxation, followed by a wash in the Krebs solution. The latter is particularly important, because several controlled studies have shown that the effectiveness of most vasodilators used to reduce intraoperative spasms is comparable to physiological sodium chloride solution [15]. It is possible that the authors, exposing ITAs to saline solution, produced a kind of washing, similar to that carried out in the present study, *in vitro*.

The use of nitroglycerin, as expected, did not affect the SM membrane potential. Nitroglycerin has a maximal effect at a constant MP, but its effectiveness has reduced proportionally to the MP increase. The findings of the present study argue with the need for a widespread use of pharmacological agents which reduce the MP, such as calcium antagonists.

5. Conclusion

The maximum potassium-induced MT of the ITA distal segments was significantly higher compared to the proximal ones. There was no statistically significant effect of phenylephrine on the degree of MT. On the contrary, epinephrine and niflumic acid cause pronounced ITA contractile response. Nitroglycerin has a marked relaxing influence on a potassium-induced spasm of the ITA distal segments. Nitroglycerin has a maximal effect at a low level of the initial MP. Therefore, if MP increases, high doses of nitroglycerin are required to achieve maximum vasodilation.

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