J. Pharm. Pharmacol. 1992, 44: 663–666
Received November 26, 1991

Effects of Isosorbide-5-mononitrate and Isosorbide-2-mononitrate on the Contractile and Electrical Activity and on the Content of Cyclic Nucleotides in Isolated Heart Muscles of the Guinea-pig and Dog

R. ATANASSOVA, G. SPASSOV*, R. BALANSKY† AND K. BOEV*

*Chemical Pharmaceutical Research Institute, Sofia 1156, Klim. Ohridski 3a, *Bulgarian Academy of Sciences-Institute of Physiology, Sofia 1113, and †National Centre of Oncology, Sofia 1156, Bulgaria*

Abstract—Isosorbide-5-mononitrate and isosorbide-2-mononitrate, the metabolites of isosorbide dinitrate, were studied for their effects on the contractile and electrical activity and on the content of cyclic nucleotides in isolated heart muscles of guinea-pig and dog. Isosorbide-5-mononitrate at all concentrations tested and isosorbide-2-mononitrate at low concentrations did not produce significant changes in the mechanical activity of guinea-pig heart preparations. Isosorbide-2-mononitrate in concentrations above 10^{-4} M inhibited the contractility and shortened the action potential of guinea-pig and dog heart muscle. Neither mononitrate had an effect on cAMP concentration but both induced a significant elevation of cGMP content in guinea-pig heart tissue, the effect of isosorbide-2-mononitrate being greater.

Organic nitrates have been used widely in the treatment of cardiovascular diseases because of their vascular smooth muscle relaxation effect. However, in addition to their peripheral vasorelaxant activity, a direct relaxant effect of nitrates on cardiac muscle and decreased inotropic function has been suggested (Brodie et al 1976; Barath et al 1981). Conversely, Bonoron-Adele et al (1981) observed positive inotropic and chronotropic effects in some angina pectoris patients after their treatment with nitrates which may be due to a direct stimulating action of these compounds on the heart muscle.

The mechanism of the direct cardiac effects of nitrates, isosorbide-5-mononitrate and isosorbide-2-mononitrate is unclear but may be related to cGMP levels in heart tissues (Schröder & Noack 1987). The influence of cyclic nucleotides on the inotropic activity has been established (George et al 1973; Sperelakis 1987), but whether the nitrate-induced electrophysiological effects on the cardiac tissue could be mediated by changes in cyclic nucleotide levels is undetermined.

The present work aimed to clarify the effects of isosorbide-5-mononitrate and isosorbide-2-mononitrate on the contractile and electrical activity of guinea-pig and dog isolated heart muscles as well as on the content of cAMP and cGMP in these tissues.

Materials and Methods

Guinea-pigs, 300–400 g, were killed by a blow on the head and their hearts removed and placed in Tyrode solution. Papillary preparations from the right ventricle not exceeding 0·7 mm in diameter, and preparations from the left atrium, not exceeding 1 mm in width, were made. These were mounted horizontally in a 2 mL bath with superfusion at a rate of 4–5 mL min^{-1}. The Tyrode solution used contained (mM): NaCl 136·9, KCl 2·68, $CaCl_2$ 2·5, $MgCl_2$ 1·15, Na_2HCO_3 11·9, NaH_2PO_4 0·42 and glucose 5·6. The solution was aerated with carbogen (95% O_2 – 5% CO_2) at a temperature of 32 ± 0·2°C (pH 7·3 ± 0·05). The muscles were attached to both a platinum stimulating electrode and to the arm of an isometric force semiconductor tensometric transducer. A second platinum stimulating electrode was placed close to the preparations. The muscles were stimulated with rectangular electrical impulses, 0·5 ms duration, amplitude—15–20% above the threshold and frequency, 2 cycles s^{-1}, using an ESU-2 electrical stimulator (USSR). The muscle contractions were registered by a recorder H-327-5 (USSR). After an equilibration period of 30 min isosorbide-5-mononitrate or isosorbide-2-mononitrate was applied in increasing concentrations ranging from 10^{-8} to 3×10^{-3} M for 10 min intervals. The changes in guinea-pig heart muscle contractility after incubation with mononitrates were expressed in percentages of contractility of the control preparations.

The effect of isosorbide-2-mononitrate (10^{-4} and 10^{-3} M) on the electrical activity of papillary preparations isolated from the right ventricle of guinea-pigs and dogs was studied according to the method of Woodbury & Brady (1956). The membrane action potentials were registered using floating glass intracellular microelectrodes with a diameter at the tip < 1 μm, filled with a 2·5 M KCl solution. An amplifier with high input impedance B-1623 (MIKI, Hungary) was used. The action potentials were recorded and photographed by means of a storage oscilloscope (Tektronix 434 (USA)) and 35 mm camera (Praktica L (Germany)). The action potential duration at 25% (APD25), 50% (APD50) and 90% (APD90) repolarization was determined.

The effect of isosorbide-2-mononitrate (10^{-5}–10^{-3} M) added to the incubation solution for 10 min intervals on

Correspondence: R. Atanassova, Chemical Pharmaceutical Research Institute, Sofia 1156, Klim. Ohridski 3a, Bulgaria.

barium chloride (10^{-4} M)-induced arrhythmia in unstimulated atrial and ventricular preparations of guinea-pig heart was studied.

The in-vivo effects of isosorbide-2-mononitrate (20 or 50 mg kg^{-1}) and isosorbide-5-mononitrate (20 mg kg^{-1}) on the cAMP and cGMP concentrations in guinea-pig cardiac tissue were also measured. The animals were injected intraperitoneally with 1–1·5 mL solution of the mononitrates dissolved in saline. Control animals were treated with the solvent under the same experimental conditions. Animals were killed and the hearts were frozen immediately in liquid nitrogen. Homogenates (15%) were prepared in 6% trichloroacetic acid by means of Potter-Evelhejm homogenizer with teflon mortar (Glenco, USA). After centrifugation (8000 *g*, 10 min) the supernatants were extracted five times with water-saturated diethyl ether. cAMP and cGMP concentrations were determined radioimmunologically using kits, supplied by Amersham, UK. The radioactivity of the samples dissolved in scintillation liquid Unisolve-I (Koch-Light, UK) was measured in a liquid scintillation counter LKB-Rackbeta (Finland). The results were expressed in pmol cyclic nucleotide (mg tissue)$^{-1}$ (wet weight).

The statistical analysis of the data obtained was carried out using analysis of variance or the Student–Fisher's *t*-test. Isosorbide-5-mononitrate and isosorbide-2-mononitrate, synthesized at the Chemical Pharmaceutical Research Institute in Sofia (Bulgaria) and barium chloride (Merck, Darmstadt), dissolved in distilled water, were used.

Results

Control experiments on the contractility of guinea-pig papillary preparations isolated from the right ventricle and from the left atrium showed that a gradual decrease of the muscle contractions occurred during the 120 min observation period (Tables 1, 2).

In-vitro experiments did not reveal any changes in the muscle mechanical activity of guinea-pig heart, atrial and ventricular preparations after applying isosorbide-5-mononitrate (10^{-8}–3×10^{-3} M). The cumulative isosorbide-5-mononitrate curves were identical to the control curves (Tables 1, 2).

In addition, no substantial changes were found in the contractility of either type of preparation after treatment with low concentrations of isosorbide-2-mononitrate (10^{-8} and 3×10^{-8} M). However, the contraction amplitude of the

Table 1. Decrease in contractility (%) of isolated muscles from the left atrium of guinea-pig heart, incubated with mononitrates for 10 min intervals.

Controls	Time (min)	Treatment: Isosorbide-5-mononitrate	Treatment: Isosorbide-2-mononitrate	Concn (M)
0·00±0·00	1	0·45±0·45	0·00±0·00	10^{-8}
2·16±1·20	10	3·29±1·81	1·22±0·26	3×10^{-8}
6·06±1·41	20	6·10±1·56	6·99±1·05	10^{-7}
8·03±1·61	30	6·28±1·33	9·05±2·42	3×10^{-7}
9·02±1·82	40	8·51±1·02	10·41±1·73	10^{-6}
10·93±2·29	50	9·42±1·41	12·76±1·77	3×10^{-6}
11·41±2·07	60	11·45±1·12	14·06±1·60*	10^{-5}
12·78±2·29	70	12·10±1·57	17·01±2·47*	3×10^{-5}
14·72±1·73	80	15·05±2·14	21·35±1·53**	10^{-4}
16·06±2·17	90	16·95±1·99	29·05±1·79**	3×10^{-4}
17·94±1·98	100	18·26±2·32	37·67±2·41**	10^{-3}
18·98±2·50	110	20·34±2·76	45·59±1·57**	3×10^{-3}

Results are expressed as mean±s.e.m., (n=9) in each experiment. * Significantly different from controls at $P<0·05$. ** Significantly different from controls at $P<0·001$.

Table 2. Contractility (%) of papillary preparations from the right ventricle of guinea-pig heart, incubated with mononitrates for 10 min intervals.

Controls	Time (min)	Treatment: Isosorbide-5-mononitrate	Treatment: Isosorbide-2-mononitrate	Concn (M)
0·00±0·00	1	0·00±0·00	0·00±0·00	10^{-8}
2·45±0·91	10	2·15±0·32	1·63±1·46	3×10^{-8}
5·36±0·83	20	6·12±1·17	6·38±1·26	10^{-7}
8·29±1·75	30	9·34±1·05	10·62±2·54	3×10^{-7}
13·95±2·10	40	12·87±1·67	16·57±2·34	10^{-6}
16·80±2·23	50	14·66±2·37	20·01±2·21*	3×10^{-6}
19·17±1·95	60	18·12±1·23	22·65±2·96*	10^{-5}
22·07±2·28	70	24·91±2·37	25·74±2·47**	3×10^{-5}
23·39±2·17	80	25·65±2·68	28·97±2·54**	10^{-4}
26·10±2·01	90	27·33±2·90	35·64±1·82**	3×10^{-4}
28·99±1·64	100	29·90±3·21	48·08±2·25**	10^{-3}
30·27±2·21	110	21·34±2·36	51·76±1·47**	3×10^{-3}

Results are expressed as mean±s.e.m., (n=9) in each experiment. * Significantly different from controls at $P<0·05$. ** Significantly different from controls at $P<0·001$.

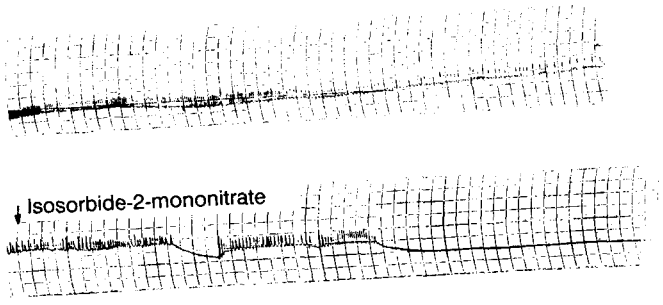

FIG. 1. Inhibition of barium chloride-induced arrhythmia in preparations from the right ventricle of guinea-pig heart by 10^{-4} M isosorbide-2-mononitrate.

isolated muscles showed a tendency to decrease after addition of this compound at higher concentrations (10^{-7}–3×10^{-5} M). The highest significant changes were obtained after applying isosorbide-2-mononitrate at concentrations exceeding 10^{-4} M (Tables 1, 2). Thus, isosorbide-2-mononitrate (10^{-4} M) caused a 6·63% decrease of the atrial muscle contractility and 5·58% decrease of the ventricular contractile activity, and at 3×10^{-3} M this mononitrate decreased these values by 26·61 and 25·86%, respectively.

In some series of experiments, a persistent arrhythmic activity of the muscle contractions was observed after the equilibration period. In these cases, the addition of isosorbide-2-mononitrate ($> 3 \times 10^{-5}$ M) removed the registered arrhythmic contractions.

The effect of isosorbide-2-mononitrate (10^{-5}–10^{-3} M) on barium chloride-induced arrhythmia in preparations of guinea-pig heart was also studied. The recordings of mechanical activity of such preparations (Fig. 1) revealed a decreased frequency of the arrhythmic contractions after the administration of this mononitrate.

The isosorbide-2-mononitrate effects on the electrical activity of preparations isolated from guinea-pig or dog right cardiac ventricle are shown in Table 4. A sharp shortening of the action potential, without significant changes in the depolarization phase was observed after addition of effective concentrations of isosorbide-2-mononitrate (10^{-4} and 10^{-3} M) for 15 min. The prolonged washing (20 min) of the preparations with Tyrode solution did not restore the action potentials of the cardiac muscle cells.

Table 3. Effects of isosorbide-5-mononitrate and isosorbide-2-mononitrate on cAMP and cGMP levels in guinea-pig cardiac tissue.

		Cyclic nucleotide levels		
	Time	cAMP	cGMP	Increase of cGMP
Controls	(min)	0·291 ± 0·017	0·025 ± 0·003	(%)
Isosorbide-2-mononitrate				
20 mg kg^{-1}	1	0·263 ± 0·021	0·073 ± 0·005	+192
	15	0·311 ± 0·050	0·083 ± 0·006	+232
	30	0·305 ± 0·029	0·104 ± 0·012	+316
50 mg kg^{-1}	1	0·331 ± 0·024	0·042 ± 0·004	+68
	15	0·255 ± 0·011	0·080 ± 0·006	+220
	30	0·322 ± 0·017	0·067 ± 0·006	+168
Isosorbide-5-mononitrate				
20 mg kg^{-1}	1	0·273 ± 0·021	0·052 ± 0·004	+108
	15	0·341 ± 0·013	0·062 ± 0·005	+148
	30	0·282 ± 0·026	0·076 ± 0·005	+204

Results are expressed as mean ± s.e.m., n = 9 in each experiment. All cGMP values were significantly different from controls ($P < 0·001$).

An attempt to clarify the possible involvement of cyclic nucleotides in the effects observed indicated that isosorbide-2-mononitrate (20 and 50 mg kg^{-1}) and isosorbide-5-mononitrate (20 mg kg^{-1}) injected intraperitoneally did not induce significant changes in cAMP content in guinea-pig cardiac tissues when compared with the control data (Table 3). However, the cGMP concentration was significantly elevated (Table 3). In this case, the effect of isosorbide-2-mononitrate was greater than that of isosorbide-5-mononitrate; the highest elevation of cGMP content was detected 30 min after the administration of isosorbide-2-mononitrate (20 mg kg^{-1}).

Discussion

The data obtained in the present study showed that isosorbide-2-mononitrate, but not isosorbide-5-mononitrate, inhibited in a dose-dependent manner cardiac muscle contractility. Both atrial and ventricular muscle preparations were sensitive to the effect of isosorbide-2-mononitrate when concentrations higher than 10^{-4} M were applied. These concentrations are much higher than plasma levels achieved in man after therapeutic doses of mononitrates (Chasseaud & Taylor 1981; Thadani et al 1987). The differences between the effects of isosorbide-2-mononitrate and isosorbide-5-mononitrate on cardiac muscle contractility may be linked to the position of the nitrate group (Kukovetz et al 1987; Schröder & Noack 1987).

Recently, a suggestion was made that inhibitory effects of nitrates on cardiac contractility are mediated by changes in cyclic nucleotide concentrations (Abiko et al 1986). The data obtained in the present study indicated that neither mononitrate tested had an effect on cAMP content in guinea-pig heart tissue. Other authors have also found no changes in cAMP concentration after in-vivo and in-vitro treatment of blood vessels with nitrovasodilators (Kobayashi et al 1980; Galvas & Di Salvo 1983; Kaufmann et al 1986). However, both compounds tested increased cGMP levels, the isosorbide-2-mononitrate being more effective than isosorbide-5-mononitrate.

Thus, changes in cGMP concentration might be involved in the pharmacological effects of mononitrates on cardiac muscle. This, however, cannot be a direct effect as although both compounds affected cGMP levels, only isosorbide-2-mononitrate influenced cardiac muscle contractility. The

Table 4. Effect of isosorbide-2-mononitrate on the action potentials of muscle cells from guinea-pig and dog right ventricles.

		APD25 (ms)	APD50 (ms)	APD90 (ms)
Guinea-pig	Control	122·0±9·1	180·3±11·0	229·5±12·5
	Isosorbide-2-mononitrate (10^{-3} M)	82·0±4·4**	138·0±8·8**	202·5±10·7**
Dog	Control	235·5±8·3	350·3±8·7	420·3±12·4
	Isosorbide-2-mononitrate (10^{-4} M)	148·5±5·9**	221·5±10·1**	334·8±12·3**

APD25, APD50 and APD90, action potential duration at 25, 50 and 90% repolarization, respectively. Data are presented as mean ± s.e.m., (n = 4) in each experiment. ** Significantly different from controls at $P < 0.001$.

ability of some substances, such as sodium nitroprusside, acetylcholine and prostaglandin $F_{2\alpha}$ to raise cGMP levels in cardiac tissue by activating guanylate cyclase has been considered by some authors as a possible explanation for their negative inotropic effects (George et al 1973; Sperelakis 1987). Bkaily & Sperelakis (1985) and Mehegan et al (1985) also showed that cGMP had a direct effect on cardiac electrical and mechanical activity, probably due to an inhibitory effect on slow calcium channels and decreased influx of calcium ions into the cells.

The data presented suggest that isosorbide-2-mononitrate could exert an antiarrhythmic effect on barium chloride-induced arrhythmia in isolated cardiac muscles. These data support clinical observations showing a favourable effect of organic nitrates on some arrhythmias probably due to their haemodynamic activity as well as to a putative effect on the myocardium (Knoebel et al 1975; Dashkoff et al 1976; Probst et al 1981).

References

Abiko, J., Fukushi, J., Haga, N., Matsumura, H. (1986) Attenuation by isosorbide dinitrate of coronary occlusion-induced acidosis in the dog myocardium. Br. J. Pharmacol. 87: 427–432

Barath, P., Kekes, E., Strausz, J. (1981) Acute global ischaemia and nitroglycerine-induced changes of the contraction-relaxation and diastolic properties of isolated rabbit heart. Acta Physiol. Pol. 32: 637–649

Bkaily, G., Sperelakis, N. (1985) Injection of cyclic GMP into heart cells blocks the slow action potentials. Am. J. Physiol. 248: H745–H749

Bonoron-Adele, S., Tariosse, L., Bricaud, H., Besse, P. (1981) Demonstration of a real inotropic effect of nitrites on myocardial contractility during anoxia and reoxygenation: attempt to determine action site with the contribution of calcium antagonistic compounds. In: Lichtlen, P., Engel, H.-J., Schrey, A., Swan, H. (eds) Nitrates III. Cardiovascular Effects. Springer-Verlag, Berlin, pp 151–170

Brodie, A., Chuck, L., Klausner, S., Grossman, W., Parmley, W. (1976) Effects of sodium nitroprusside and nitroglycerin on tension prolongation of cat papillary muscle during recovery from hypoxia. Circ. Res. 39: 596–601

Chasseaud, L., Taylor, T. (1981) Pharmacokinetics of isosorbide mononitrates in human subjects. In: Lichtlen, P., Engel, H.-J., Schrey, A., Swan, H. (eds) Nitrates III. Cardiovascular effects. Springer-Verlag, Berlin, pp 47–54

Dashkoff, N., Roland, J., Varghese, P., Pitt, B. (1976) Effect of nitroglycerine on ventricular fibrillation threshold of non-ischemic myocardium. Am. J. Cardiol. 38: 184–188

Galvas, P., Di Salvo, J. (1983) Concentration and time-dependent relationship between ISDN-induced relaxation and formation of cyclic GMP in coronary arterial smooth muscle. J. Pharmacol. Exp. Ther. 224: 373–378

George, W., Wilkerson, R., Kadowitz, P. (1973) Influence of acetylcholine on contractile levels in the isolated perfused rat heart. Ibid. 184: 228–235

Kauffman, R., Schenck, K., Gonery, B., Cohen, M. (1986) Effects of pinacidil on serotonin-induced contractions and cyclic nucleotide levels in isolated rat aortae: comparison with nitroglycerin, minoxidil and hydralazine. J. Cardiovasc. Pharmacol. 8: 1195–1200

Knoebel, S., Rasmussen, S., Noble, R., Mihalick, M. (1975) Nitroglycerine and premature ventricular complexes in myocardial infarction. Br. Heart J. 37: 1064–1068

Kobayashi, A., Suzuki, Y., Kamikawa, T., Hayashi, H., Yamasaki, N. (1980) The effects of nitroglycerine on cyclic nucleotides in the coronary artery in vivo. Life Sci. 27: 1679–1685

Kukovetz, W., Holzmann, S., Romanin, C. (1987) Mechanism of vasodilation by nitrates: role of cyclic GMP. In: Mononitrates. Proc. Int. Symp., London, Cardiology 74 (Suppl. 1): 12–19

Mehegan, J., Muir, W., Unverforth, D. (1985) Electrophysiological effects of cyclic GMP on canine cardiac Purkinje fibres. J. Cardiovasc. Pharmacol. 7: 30–35

Probst, P., Schwarzer, C., Pachinger, O. (1981) The action of nitroglycerine on pacing-induced arrhythmias. In: Lichtlen, P., Engel, H.-J., Schrey, A., Swan, H. (eds) Nitrates III. Cardiovascular Effects. Springer-Verlag, Berlin, pp 377–383

Schröder, H., Noack, E. (1987) Structure-activity relationship of organic nitrates for activation of guanylate cyclase. Arch. Int. Pharmacodyn. Ther. 290: 235–247

Sperelakis, N. (1987) Regulation of calcium slow channels of cardiac and smooth muscles by adenosine nucleotides. In: Cardiac Electrophysiology and Pharmacology of Adenosine and ATP: Basic and Clinical Aspects. Alan Press Inc., New York, pp 135–193

Thadani, U., Prasad, R., Hamilton, S., Voyles, W., Doyle, R., Karpow, S., Reder, R., Teague, S. (1987) Isosorbide-5-mononitrate in angina pectoris: plasma concentrations and duration of effects after acute therapy. Clin. Pharmacol. Ther. 42: 58–65

Woodbury, J., Brady, A. (1956) Intracellular recording from moving tissue with a flexibly mounted ultramicroelectrode. Science 123: 100–101