Acta Poloniae Pharmaceutica – Drug Research, Vol. 67 No. 5 pp. 537–542, 2010 ISSN 0001-6837
Polish Pharmaceutical Society

ANTIARRHYTHMIC ACTIVITY OF NOVEL S-ENANTIOMERS OF PYRROLIDIN-2-ONE DERIVATIVES WITH ADRENOLYTIC PROPERTIES

JACEK SAPA

Department of Pharmacodynamics, Laboratory of Pharmacological Screening, Faculty of Pharmacy, Medical College, Jagiellonian University, Medyczna 9, 30-688 Kraków, Poland

Abstract: A six new analogs of MG-1(S), 1-[2-hydroxy-3-(4-phenyl-1-piperazinyl)propyl]-pyrrolidin-2-one, with adrenolytic properties were tested for electrocardiographic and antiarrhythmic activity in model ventricular arrhythmias associated with coronary artery occlusion and reperfusion in the non-working isolated perfused rat heart and additionally in barium chloride – induced arrhythmia *in vivo*. All tested compounds slightly decreased the heart rate, prolonged P-Q, Q-T intervals and QRS complex. The antiarrhythmic effects of all tested compounds were weaker than the reference compound MG-1(S).

Keywords : pyrrolidin-2-one derivatives, α-adrenoceptor blocking activity, antiarrhythmic, occlusion and reperfusion

Our earlier research showed that racemic mixture MG-1(R,S), 1-[2-hydroxy-3-(4-phenyl-1-piperazinyl)propyl]-pyrrolidin-2-one and its S-enantiomer, significantly decreased systolic and diastolic blood pressure, and possessed antiarrhythmic activity but the highest effect was shown by S-enantiomer. R-enantiomer did not show antiarrhythmic and hypotensive activity. S-enantiomer of MG-1 statistically diminished arrhythmias associated with coronary artery occlusion and reperfusion in isolated rat hearts. This compound had affinity for α_1 – and α_2 – adrenoceptors and antagonized the pressor response elicited by epinephrine and methoxamine, (1–4).

As a continuation of this study, 6 new analogs of S-enantiomer of MG-1 with adrenolytic properties (active in adrenaline-induced arrhythmia) (4), were tested in model ventricular arrhythmias associated with coronary artery occlusion and reperfusion in the non-working isolated perfused rat heart and additionally in barium chloride-induced arrhythmia *in vivo*.

EXPERIMENTAL

Animals

The experiments were carried out on male Wistar rats (180–250 g). Animals were housed in constant temperature facilities exposed to 12:12 h light-dark cycle and maintained on a standard pellet diet and tap water given *ad libitum*. All procedures were according to the Animal Care and Use Committee Guidelines, and approved by the Ethical Committee of Jagiellonian University, Kraków. Control and experimental groups consisted of 6–8 animals each.

Drugs and compounds

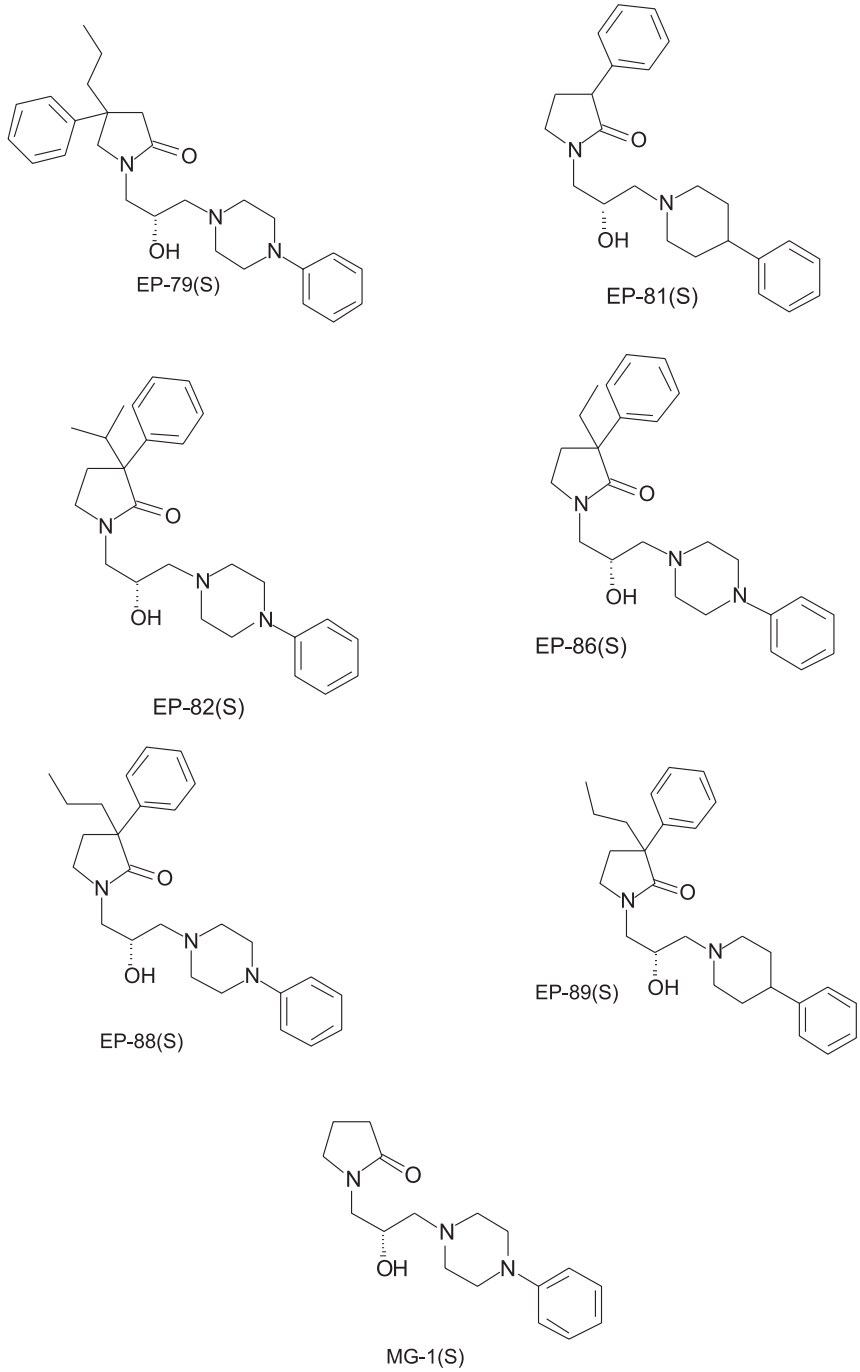
All tested compounds (Scheme 1) were synthesized in the Department of Physicochemical Drug Analysis, Pharmaceutical Faculty, Jagiellonian University. Synthesis of tested compounds was described previously (4).

Barium chloride (POCh, Poland), sodium heparin (Polfa), thiopental sodium (Biochemie GmbH, Vienna), diphenylhydantoin sodium salt (Epanutin, Parke-Davis), quinidine (Sigma Aldrich).

Ventricular arrhythmias associated with coronary artery occlusion and reperfusion in the non-working isolated perfused rat heart

Hearts from thiopental-anesthetized (45–60 mg/kg, *ip*) rats were perfused according to the Langendorff technique (4–6), at constant pressure of 70 cm H_2O (6.87 kPa) with Chenoweth-Koelle solution continuously gassed with 95% O_2 plus 5% CO_2

* Corresponding author: jaceksapa@interia.pl



Scheme 1. Structure of tested compounds and MG-1(S)

of the following composition (mmol/l): NaCl (120.0), KCl (5.6), $MgCl_2$ (2.2), $NaHCO_3$ (19.0), $CaCl_2$ (2.4), and glucose (10.0).

The effect of tested compounds, in concentration of 10^{-8} to 10^{-4} M, on coronary flow (cardiac effluent), electrocardiogram (obtained by two stainless steel electrodes, one inserted into the muscle of the ventricular wall and another attached to the metal aortic cannula) were assessed after 15–20 min of initial stabilization.

Non-working isolated hearts were mounted as described above for recording coronary flow and ECG. After a 15 min stabilization period, acute regional myocardial ischemia was produced for 15 min by installing a clip on the left coronary artery close to its origin (ischemic period). The clip was then reopened, and changes during reperfusion were monitored for 30 min (reperfusion period). Occlusion and reperfusion were verified by measuring coronary flow before occlusion, after occlusion and after reperfusion.

Ligation of the coronary artery resulted in 24–28% reduction in coronary flow. Reperfusion was followed by a return of the coronary flow. Reperfusion induced arrhythmias manifested by ventricular premature beats (VBs), ventricular tachycardia (VT) and ventricular fibrillation (VF).

Electrocardiograms (ECGs) were analyzed according to the guidelines of the Lambeth Conventions for VBs, bigeminy, salvos (less than 4 successive VBs), VT (4 or more successive VBs and VF.

In order to obtain a measure for the intensity of the arrhythmias, an Arrhythmia Severity Index was calculated for each heart according to Bernauer (7). The following values were attributed: occurrence of up to 10 ventricular extrasystoles during the first 30 min of reperfusion – 1, more than 10 – 2, VT or ventricular flutter – 3, and VF – 4. Bigeminy and salvos were not quantified separately but included with VBs.

Agents were added to the perfusion medium 15 min before coronary artery ligation and the concentration was maintained for the rest of the perfusion period.

Prophylactic antiarrhythmic activity in barium chloride-induced arrhythmia (8)

Barium chloride solution was injected into the caudal vein of rat (32 mg/kg, in a volume of 1 mL/kg). The tested compounds were given *i.v.* 15 min before arrhythmogen. The criterion of antiarrhytmic activity was a gradual disappearance of arrhythmia and restoration of sinus rhythm.

Statistical analysis

The data are expressed as the means + SEM. The data were evaluated by one-way analysis of variance (ANOVA) followed by Duncan test; $p < 0.05$ was considered significant.

RESULTS

Ventricular arrhythmias associated with coronary artery occlusion and reperfusion in the non-working isolated perfused rat heart

During the 30 min period of coronary artery reperfusion, all hearts in the control group developed VBs. The incidence of VT and VF was 60 and 50%, respectively (Table 1).

Compared with control hearts, all tested compounds not significantly diminished the incidence of VT and VF. The compounds reduced the incidence of VT and VF at a concentration 10^{-5} M. At this concentration their arrhythmias severity index was similar and had a value about 4.5 – 4.8 (control hearts 5.4) (Table 1). This effect was weaker comparatively to MG-1(S). In previous studies compound MG-1(S) significantly diminished the incidence of VT and VF. In concentration 10^{-6}–10^{-5} M it significantly reduced the incidence of VBs (about 50–20%), VT (about 90–80%) and VF (15%). The arrhythmias severity index was lower (2.1 – 2.9), compared with control hearts (Table 1).

Reference drugs in this model; phenytoin and quinidine (I class Vaughan Williams classification) (8) showed significantly antiarrhythmic effect in this test in concentrations 10^{-5} M (phenytoin) and 10^{-6}–5×10^{-6} M (quinidine) (Table 1).

Influence of tested compounds on ECG parameters on the non-working isolated perfused rat heart

The six tested compounds (EP-79(S), EP-81(S), EP-82(S), EP-86(S), EP-88(S) and EP-89(S)) decreased the number of cardiac beats per minute, prolonged P-Q, Q-T, intervals and QRS complex.

All compounds in concentration 10^{-6} – 10^{-4} M significantly decreased the number of cardiac beats per minute (10^{-6} M: 9–14%; 10^{-4} M: 17–22%). This chronotropic effect was similar to phenytoin (10^{-6} M: 11%; 10^{-4} M: 21%) and weaker than quinidine (10^{-7} M – 10^{-4} M: 17–35%) (Fig. 1).

The tested compounds in concentration 10^{-6} – 10^{-4} M significantly prolonged P-Q intervals (10^{-6} M: 14–25%; 10^{-4} M: 27–39%), Q-T intervals (10^{-6} M: 12–27%; 10^{-4} M: 24–39%) and QRS complex (10^{-6} M: 12–17%; 10^{-4} M: 23–31%), (Figs. 2–4). The electrocardiographic changes observed after administration of the tested compounds were similar to those observed for quinidine but weaker. Phenytoin similarly prolonged P-Q and Q-T interval, but did not change the QRS complex, (Figs. 1–4). The obtained results suggest that the new pirrolidin-2-one derivatives possess „quinidine-like" properties.

Prophylactic antiarrhythmic activity in barium chloride-induced arrhythmia

For examined rats, intravenous injections of high dose of barium chloride (32 mg/kg) caused a rapid ventricular extrasystoles and VF in all animals (100%), that led to death within 3–8 min. The tested

Table 1. Effect of tested compounds on reperfusion-induced arrhythmias.

Compound	Concentration (M)	VBs incidence (%)	Bigeminy incidence (%)	Salvos incidence (%)	VT incidence (%)	VF incidence (%)	Arrhytmias severity index
Control	-	100	50	30	60	50	5.4 ± 0.6
EP-79(S)	10^{-6}	100	60	20	50	30	4.9 ± 0.7
	10^{-5}	80	30	20	50	25	4.6 ± 1.2
	10^{-4}	75	50	25	50	75	5.5 ± 0.6
EP-81(S)	10^{-6}	100	60	20	60	80	6.0 ± 0.4
	10^{-5}	50	50	30	60	40	4.8 ± 1.2
	10^{-4}	80	50	25	50	75	5.5 ± 0.3
EP-82(S)	10^{-6}	100	40	20	60	40	5.0 ± 0.5
	10^{-5}	100	50	20	60	20	4.5 ± 0.3
	10^{-4}	100	20	20	60	30	4.6 ± 0.4
EP-86(S)	10^{-6}	100	60	20	60	80	6.0 ± 0.6
	10^{-5}	50	100	40	60	40	4.8 ± 0.8
	10^{-4}	100	50	30	60	40	5.0 ± 0.3
EP-88(S)	10^{-6}	100	60	40	62.5	37.5	5.0 ± 0.2
	10^{-5}	100	25	25	62.5	25	4.5 ± 0.8
	10^{-4}	100	30	30	75	25	5.0 ± 0.5
EP-89(S)	10^{-6}	60	50	30	60	40	4.8 ± 0.6
	10^{-5}	100	40	30	50	40	4.8 ± 0.3
	10^{-4}	80	50	40	40	60	5.4 ± 1.1
MG-1(S)	10^{-6}	50	25	0	10	10	2.1 ± 0.4 ***
	10^{-5}	80	20	0	20	10	2.9 ± 0.8*
	10^{-4}	100	50	17	33	33	4.0 ± 0.9
PHENYTOIN	10^{-6}	100	0	0	75	0	4.3 ± 0.3
	10^{-5}	100	0	0	25	0	2.6 ± 0.4**
	10^{-4}	100	25	0	40	20	4.1 ± 0.7
QUINIDINE	10^{-6}	83.3	16.7	0	50	0	2.3 ± 0.8 *
	5 x 10^{-6}	16.7	0	0	33.3	0	1.2 ± 0.6****

Each value was obtained from 6–8 hearts. Significantly different *vs*. control: **** $p < 0.001$, *** $p < 0.01$, ** $p < 0.02$, * $p < 0.05$.

compounds were given intravenously 15 min before arrhythmogen.

The highest activity was demonstrated by reference compound MG-1(S)**,** which reduced incidence of VF (by 20–60%) in doses 5, 10 and 20 mg/kg. At these doses it protected the animals against death, too (by 10–60%). The ED_{50} value of compound MG-1(S) was 10.4 mg/kg, (Table 2).

Compounds: EP-79(S), EP-81(S), EP-82(S), EP-86(S), EP-88(S) and EP-89(S) possessed weak antiarrhythmic properties, reduced incidence of ventricular fibrillation in 10–33% in a dose of 10 mg/kg and protected the animals against death by 10–20% (Table 2).

DISCUSSION

In model ventricular arrhythmias associated with coronary artery occlusion and reperfusion, compound MG-1(R,S) and its S-enantiomer significantly diminished the incidence of VT and VF. This effect was similar to that of quinidine. It was suggested that S-enantiomer had high affinity to α_1-adrenergic receptors and had the most antiarrhythmic activity. On the ground of these results, new six analogs of S-enantiomer MG-1 were investigated.
Binding studies presented in (4) showed that all these compounds [(EP-79(S), EP-81(S), EP-82(S), EP-86(S), EP-88(S) and EP-89(S)] displaced

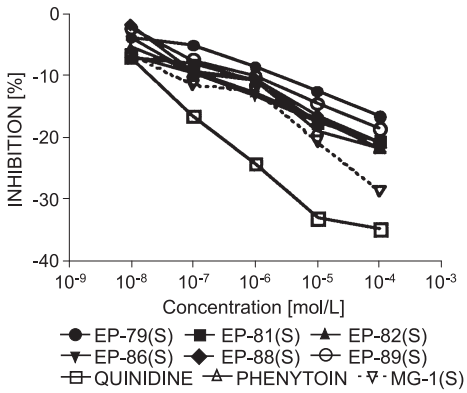


Figure 1. Influence of tested compounds on heart rate

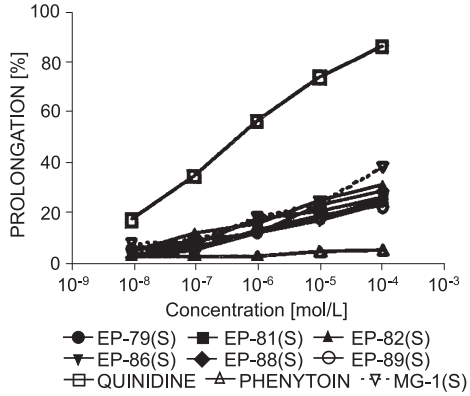


Figure 3. Influence of tested compounds on QRS complex

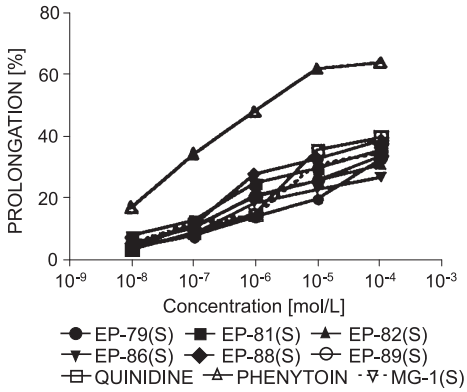


Figure 2. Influence of tested compounds on P–Q intervals

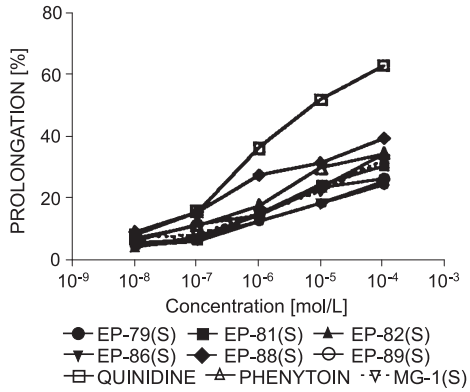


Figure 4. Influence of tested compounds on Q–T intervals

Table 2. Prophylactic antiarrhytmic activity in barium chloride-induced arrhythmia.

Compound	Dose (mg/kg)	Ventricular fibrillation reduction (%)	ED_{50} (mg/kg)	Mortality reduction (%)
EP-79(S)	10	15	-	20
EP-81(S)		33	-	10
EP-82(S)		20	-	16
EP-86(S)		12	-	10
EP-88(S)		20	-	10
EP-89(S)		10	-	16
MG-1(S)	5	20	10.4 (8.7 – 12.5)	10
	10	55		60
	15	60		40

[^{3}H]prazosin from cortical binding sites (K_i = 370–880 nM) and decreased pressor response elicited by epinephrine, norepinephrine and methoxamine. These compounds injected intravenously 15 min before adrenaline, diminished the occurrence of extrasystoles and reduced mortality (ED_{50} = 6.2 – 13.2 mg/kg) (4).

The antiarrhythmic activity of tested compounds was examined in arrhythmia associated with coronary artery occlusion and reperfusion rat mod-

els. Many authors suggest that the rat coronary artery ligation and reperfusion model can be recommended as a screen for new antiarrhythmic agents (9–13). It has been well known that α-blocking agents (phentolamine, prazosin, corynanthine, rauwolscine and yohimbine) in relatively high doses diminished or prevented reperfusion arrhytmias and cardiac arrhythmias caused by adrenaline intoxication (7, 14–17). Phentolamine, a non-selective blocker of α_1- and α_2-adrenoceptors, and the selective α_1-adrenoceptor antagonist prazosin could reduce the incidence of ischemia-induced ventricular fibrillation, which is in accordance with several studies in the ischemic or reperfused rat, cat and dog myocardium (18–25). All tested compounds had a worse effect in this model of the arrhythmia comparatively to MG-1(S), which in a concentration of 10^{-5}–10^{-6} M significantly diminished the incidence of VT and VF. The arrhythmias severity index was lower (2.1 – 2.9), compared with control hearts (5.4). The antiarrhythmic activity of MG-1(S) in this test was comparable to that reported for phenytoin and quinidine.

The electrocardiographic changes observed after administration of all tested compounds are similar to those seen after administration of MG-1(S). The changes in ECG were similar but significantly weaker that those after quinidine (8).

Only compound MG-1(S), contrary to tested compounds, administered 15 min before barium chloride in doses 10 and 15 mg/kg prevented in a statistically significant manner the occurrence of VF in 55–60%.

These results suggest that six new analogs of S 1-[2-hydroxy-3-(4-phenyl-1-piperazinyl)propyl]-pyrrolidin-2-one have weaker antiarrhythmic effect than the reference compound.

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Received: 30. 03. 2010