European Journal of Pharmacology 735 (2014) 10–16

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular pharmacology

Antiarrhythmic, hypotensive and α_1-adrenolytic properties of new 2-methoxyphenylpiperazine derivatives of xanthone

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ARTICLE INFO

Article history:
Received 29 November 2013
Received in revised form
26 March 2014
Accepted 1 April 2014
Available online 18 April 2014

Keywords:
Antiarrhythmic
Hypotensive
α_1-adrenoceptor antagonist
Xanthone derivatives
Piperazine derivatives

ABSTRACT

The main goal of this study was to assess antiarrhythmic and hypotensive activity of new 2-methoxyphenylpiperazine derivatives of xanthone. In order to better understand mechanism of action of studied compounds, their abilities to antagonize the increase in blood pressure elicited by adrenaline, noradrenaline and methoxamine, as well as the antagonistic properties for α_1-adrenoceptors on isolated rat aorta were evaluated. Therapeutic antiarrhythmic activity was investigated in an adrenaline-induced model of arrhythmia. Hypotensive activity in normotensive rats was evaluated after oral administration. Influence on blood vasopressor response and α_1-adrenoceptors in rat thoracic aorta was evaluated to determine if the observed cardiovascular effects could be related to α_1-adrenolytic properties. Tested compounds produced antiarrhythmic and hypotensive activity. The most active compound was **MH-99** – (*R,S*)-4-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride. All studied compounds showed α_1-adrenolytic properties in the *in vivo* and *in vitro* tests. The results indicate that the new valuable compounds with antiarrhythmic and hypotensive activity might be found in the group of xanthone derivatives. Further pharmacological utility of these compounds should be investigated.

1. Introduction

Cardiovascular diseases are major contributors to morbidity and mortality in developed countries. In the past decade, arrhythmias including ventricular fibrillation account for nearly one quarter of all cardiovascular-related deaths. Drugs that are currently marketed as antiarrhythmics are not uniformly effective and frequently cause adverse effects, such as bradycardia, tiredness, dizziness or thyroid dysfunction. Nevertheless, the most important of these side effects is the potential to generate new life-threatening arrhythmias (proarrhythmic effect), (Estrada and Darbar, 2008). It has been reported, that the drug-induced arrhythmia can be recognized in up to 5% of patients receiving antiarrhythmic drugs such as amiodarone, flecainide, sotalol and quinidine. In particular, pre-existent cardiac disease, bradyarrhythmias and liver disease have been identified as clinical risk factors for drug-induced arrhythmia (Petropoulou et al., 2014).

Another, well-recognized cardiovascular risk factor is hypertension. Despite the vast population of hypertensive drugs, some patients do not reach an adequate blood pressure though they use three different antihypertensive agents. Moreover, available antihypertensive as well as antiarrhythmic drugs frequently cause side effects, such as bradycardia, tiredness, sleep disturbances, changes in mood, dry mouth, blurry vision, diarrhea or impotence (Bardage and Isacson, 2000; Oliveras and de la Sierra, 2014). Therefore new potential antiarrhythmic and hypotensive agents are still urgently required for the treatment of a wide range of cardiovascular diseases.

Xanthones are a class of heterocyclic compounds, widely distributed in nature. Nowadays, xanthones and xanthone derivatives are isolated from plants or synthesized chemically. They have been shown to possess profitable effects on several cardiovascular diseases, such as hypertension, ischemic heart disease and atherosclerosis. It has been reported that xanthone derivatives possess antiarrhythmic and hypotensive (Librowski et al., 2004; Marona et al., 2009; Rapacz et al., 2011), vasorelaxant and antiplatelet (Cheng and Kang, 1997; El-Seedi et al., 2010), antithrombotic (Lin et al., 1996) and antioxidant activities (Jiang et al., 2004).

In our previous studies, we demonstrated significant prophylactic antiarrhythmic activity in adrenaline-induced arrhythmia as well as high hypotensive activity after i.v. administration of several xanthone derivatives with a 2-methoxyphenylpiperazine moiety (Szkaradek et al., 2013). Three of them, designated as **MH-94**,

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http://dx.doi.org/10.1016/j.ejphar.2014.04.010
0014-2999/

MH-99 and **MH-105**, revealed also the highest affinity for α_1-adrenoceptors in radioligand binding assay (Ki = 4, 18 and 50 nM, respectively). It is well known that sympathetic activation plays an important role in the pathogenesis of cardiac dysfunctions. Therefore, agents that antagonize adrenergic receptors are effective and widely used in the treatment of cardiovascular diseases.

Herein, we continue our pharmacological investigations in this group of xanthone derivatives. We evaluated their therapeutic antiarrhythmic activity in adrenaline-induced arrhythmia and hypotensive activity after p.o. administration. Furthermore, in order to verify that the hypotensive activity is associated with their α_1-adrenoceptor blocking properties, we evaluated their ability to antagonize the increase in blood pressure elicited by adrenaline, noradrenaline and methoxamine. Moreover, we examined their antagonistic properties for α_1-adrenoceptors on isolated rat aorta. Because the studied compounds as well as urapidil (α_1-adrenoceptors antagonist) contain the 2-methoxyphenylpiperazine moiety in their structure, we used it as a reference compound.

2. Materials and methods

2.1. Animals

All experiments were performed on normotensive male Wistar rats weighing 180–250 g (Source: Animal House, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland, stocks name KRF: WI(WU)). The animals were kept in plastic cages in a room at constant temperature of 20 ± 4 °C, under a 12/12 h light/dark cycle (light on from 7 a.m. to 7 p.m.). They had free access to food (standard laboratory pellets) and water before experiments. The control and study groups consisted of five to eight animals each. The experiments were performed between 8 a.m. and 3 p.m. The animals were killed by cervical dislocation immediately after the experiment. All the procedures were conducted according to the Animal Care and Use Committee guidelines, and approved by the Local Ethics Committee of the Jagiellonian University in Kraków (resolution no. 99/2010 and 116/2011).

2.2. Therapeutic antiarrhythmic activity in adrenaline-induced arrhythmia

Therapeutic antiarrhythmic activity was determined according to the method of Szekeres and Papp (1968). The arrhythmia was evoked in rats under anesthesia with thiopental (75 mg/kg, i.p.) by intravenous (i.v.) injection of adrenaline (20 μg/kg, in volume of 1 ml/kg). The tested compounds were administered by i.v. route at the peak of arrhythmia, immediately after administration of adrenaline. The ECG was recorded continuously for 5 min. The criterion of antiarrhythmic activity was the reduction of premature beats in comparison to the control group (Sapa et al., 2011a). The ED50 values were calculated according to the method of Litchfield and Wilcoxon (1949).

2.3. Influence on blood pressure in rats

Male Wistar normotensive rats were anesthetized with thiopental (75 mg/kg) by i.p. injection. The right carotid artery was cannulated with polyethylene tube filled with heparin in saline to facilitate pressure measurements using a Datamax apparatus (Columbus Instruments, USA), (Kubacka et al., 2013). The studied compounds were administrated orally using an intragastric probe in a wide range of doses, after a 15 min stabilization period, at a constant volume of 1 ml/kg.

2.4. Influence on blood vasopressor response in rats

The influence of studied compounds, given intravenously at the doses of 0.31 and/or 0.62 mg/kg on the increase in blood pressure elicited by adrenaline (2 μg/kg), noradrenaline (2 μg/kg) and methoxamine (150 μg/kg), was examined according to the previously described method (Kubacka et al., 2013). Adrenaline, noradrenaline and methoxamine were injected into caudal vein before administration of tested compounds (control group) and again 5 min after the studied compounds were given.

2.5. Influence on α_1-adrenoceptors in rat thoracic aorta

Isolated rat aorta was used to examine the effect on α_1-adrenoceptors according to the method previously described by Marona et al. (2011) with some minor modifications. Thoracic aorta was carefully isolated from anaesthetized rats (thiopental sodium, 75 mg/kg i.p.) and immersed in a Krebs–Henseleit solution (NaCl 119 mM, KCl 4.7 mM, $CaCl_2$ 1.9 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.2 mM, $NaHCO_3$ 25 mM, glucose 11 mM, and EDTA 0.05 mM). Then the aorta was cleaned of surrounding fat and connective tissues. Prior to this, the aorta was cut into ring preparations of about 4 mm in length, and the endothelium was removed by gently rubbing the luminal surface. Subsequently, the arterial preparation was suspended using stainless steel pins in 30 ml chambers filled with a medium at 37 °C and pH 7.4 with constant oxygenation (O_2/CO_2, 19:1). Muscle tension changes were recorded with an isometric FDT10-A force displacement transducer (BIOPAC Systems, Inc., COMMAT Ltd., Turkey). The aortic rings were equilibrated at optimal tension of 2 g for 2 h in a medium containing yohimbine (0.1 μM) and propranolol (1 μM) in order to block α_2- and β-adrenoceptors. During an equilibration period, rat aorta was exposed to 0.1 μM noradrenaline and washed every 30 min (Villalobos-Molina et al., 2002). In the last stimulation with noradrenaline, the aorta was exposed to carbachol (1 μM) to verify the functionality of endothelium. The absence of endothelium was confirmed by the lack of a relaxing response to carbachol (Furchgott and Zawadzki, 1980). Two cumulative concentration–response to phenylephrine (Phe) curves were obtained for each aortic ring in the absence and presence of antagonist by the method of Van Rossum (1963). Each arterial ring was incubated with antagonist for 30 min. In order to avoid fatigue of the arterial preparation, a 60-min recovery period was allowed between phenylephrine curves.

2.6. Data analysis

Results are expressed as mean ± S.E.M. The significance differences between mean values were calculated using GraphPad Prism 4.0 software (GraphPad Software Inc., San Diego, CA, USA) by one-way analysis of variance (ANOVA) with the post hoc Dunnett's multiple comparison test. The influence on the blood pressure was calculated by repeated measures ANOVA. Differences were considered statistically significant at $P < 0.05$.

Concentration–response curves were analyzed by non-linear regression using GraphPad Prism 4.0 software (GraphPad Software Inc., San Diego, CA, USA), as previously described by Kubacka et al. (2013). Data are the mean ± S.E.M. of 4–8 separate experiments. Schild analysis was performed to determine the pA_2 values (Arunlakshana and Schild, 1997).

The log-probit method described by Litchfield and Wilcoxon (1949) was used to establish median effective doses (ED_{50}) for compounds in the adrenaline-induced model of arrhythmia.