ACTA BIOLOGICA CRACOVIENSIA Series Zoologia 53: 63–67, 2011

Effect of New Xanthone Derivatives on the Level of Endogenous Nitric Oxide in Some Tissue Homogenates in Rats

Anna Rapacz*[1], Karolina Pytka*, Magdalena Dudek*, Tadeusz Librowski*, Kinga Sałat*, Barbara Filipek*, Henryk Marona** and Natalia Szkaradek**

** Department of Pharmacodynamics, Jagiellonian University, Faculty of Pharmacy, Medical College, Medyczna 9, 30-688 Cracow, Poland*
*** Chair of Organic Chemistry, Department of Bioorganic Chemistry, Jagiellonian University, Medical College, Medyczna 9, 30-688 Cracow, Poland*

Accepted August 31, 2011

Nitric oxide (NO) is synthesized from L-arginine by NO synthase (NOS) in endothelial cells. It has been found that the levels of endogenous inhibitors of NOS, which inhibit NO synthesis, are significantly increased in animals and patients with atherosclerosis and hypertension. Free NO is known to be involved in the central and peripheral regulation of cardiovascular function. In our previous research we examined a series of new aminoalcanolic derivatives of xanthone, which had high antiarrhythmic and/or hypotensive activity and high affinity for α/β_1-adrenoceptors. We suppose that hypotensive activity could be connected with α/β_1-adrenoceptor blocking properties. The aim of the present study was to examine the possible involvement of other mechanisms in the observed hypotensive properties so we assessed the involvement of the most active xanthone derivatives in the NO pathway. We measured the influence of new xanthone derivatives on the level of endogenous nitric oxide in some tissue homogenates using Griess's method. However, none of the tested compounds significantly increased the NO level in the heart, liver, kidney or brain tissues, as compared with the control. The results indicate that the mechanism of the hypotensive action of the examined new xanthone derivatives is not connected with the release of endogenous NO.

Key words: xanthone, hypotensive activity, nitric oxide, NOS

INTRODUCTION

Xanthones are a class of heterocyclic compounds, widely distributed in nature. Nowadays, xanthones and xanthone derivatives are isolated from plants or synthesized chemically. They exhibit a variety of biological activities, including anti-inflammatory, anti-platelet aggregation, antithrombotic, vasorelaxant, antitumor and antimicrobial activities. The pharmacological properties of xanthones in the cardiovascular system have attracted great interest. Xanthones and xanthone derivatives have

[1] rapacz.anna@gmail.com

PL ISSN 0001-530X

been shown to possess beneficial effects on some cardiovascular diseases, including ischemic heart disease, atherosclerosis, hypertension and thrombosis. Xanthonoids isolated from Gentianaceous plants have been proved to be potent inhibitors of platelet aggregation and vasoconstriction (Lin et al., 2009). Moreover, a series of synthetic xanthone derivatives have been shown to possess high hypotensive activity in rats (Marona et al., 2008; Wang et al., 2002). Among many interesting properties, xanthones show strong antioxidant activity due to the presence of hydroxy groups and/or a catechol moiety at key position. Oxygenated and prenylated xanthones, extracted from mango, have been shown to be powerful antioxidants in animals and humans (Santos et al., 2011). Inhibition of endogenous nitric oxide synthase (NOS) inhibitors by xanthones may be responsible for enhancing endothelium function and reduction of events associated with atherosclerosis (Jiang et al., 2004).

In our previous research we examined a series of new aminoalcanolic derivatives of xanthone, which had high antiarrhythmic and/or hypotensive activity and high affinity for α/β_1-adrenoceptors (Marona et al., 2008a, 2009). We suppose that hypotensive activity could be connected with α/β_1-adrenoceptor blocking properties. It is known that most classic β-blockers, classified according to Vaughan Williams as second-class antiarrhythmic drugs, and hypotensive drugs contain a 1-aroxy-3-alkylamino-2-propanol group in their structure (propranolol, atenolol, metoprolol, acebutolol). Furthermore, the 1-aroxy-3-alkylamino-2-propanol group is a structural element of some third-generation nonselective β-blockers with additional α-adrenolytic properties (carvedilol) which have beneficial blood-pressure-lowering effects. Moreover, nebivolol, a third-generation β-blocker, offers a unique mix of β_1-selectivity and NO-mediated vasodilatation. Nebivolol is a racemic mixture of L-nebivolol and D-nebivolol. The D-isomer appears to be responsible for the β-blocking effects of the drug, while the L-isomer stimulates endothelial nitric oxide synthase (eNOS) without having a significant effect on β_1-receptors at therapeutic doses. Although the mechanisms responsible for the drug actions on nitric oxide are controversial, several theories have been suggested, including agonistic effects on β_3-receptors, stimulation of endothelial adenosine triphosphate efflux and activation of eNOS via binding to β_2-receptors (Dery et al., 2011).

The aim of the present study was to examine the possible involvement of other mechanisms in the observed hypotensive properties. For that purpose we assessed the involvement of the most active xanthone derivatives in the NO pathway. Free NO is known to be involved in the central and peripheral regulation of cardiovascular function (Hink et al., 2003; Napoli and Ignarro, 2009; Sy et al., 2001).

MATERIAL AND METHODS

Drugs

The investigated xanthones MH-82 (5-(3*N*-(2-amino-2-methyl-1-hydroxypropyl)-2-hydroxypropoxy)-3-chloro-9*H*-xanthen-9-one hydrochloride), MH-87 (4-(3-(Allylamino)-2-hydroxypropoxy)-9*H*-xanthen-9-one hydrochloride), MH-89 (2-(3-(Allylamino)-2-hydroxypropoxy)-9*H*-xanthen-9-one hydrochloride) and MH-97 (4-(3*N*-(2-Amino-2-methyl-1,3-dihydroxypropyl)-2-hydroxypropoxy)-9*H*-xanthen-9-one hydrochloride) were synthesized at the Chair of Organic Chemistry, Department of Bioorganic Chemistry, Jagiellonian University, Medical College in Cracow (Marona et al., 2009). The chemicals used were: Heparin sodium (Heparinum, Polfa S.A., Poland), Tiopental sodium (Thiopental Natrium, HEFA-Frenon Arzeimittel, Germany), Sulfanilamide (Sigma-Aldrich, Germany), N-(1-naphtyl)-ethylenediamine (Sigma-Aldrich, Germany), Folin-Ciocalteau reagent (Sigma-Aldrich, Germany); other chemicals used were obtained from Polish Reagent Company (P.O.C.H., Poland). All the chemicals were of pro analysis grade. The investigated and reference compounds were suspended in saline.

Animals

The animals used for the tests came from the Animal Breeding Farm of the Faculty of Pharmacy, Medical College, Jagiellonian University. Wistar albino male rats weighing 150-200 g were used for the tests. The animals were housed and fed in a laboratory and kept at constant temperature of 22°C under standard conditions with natural light-dark cycles. Each experimental group consisted of 4-6 animals and all the animals were

used only once. The treatment of the laboratory animals used in the present study was in full accordance with the respective Polish and European regulations and was approved by the Local Ethics Committee.

Statistical analysis

All the data are expressed as mean ± SEM. The level of statistical significance was determined by analysis of variance (ANOVA test). Differences were considered significant when $p < 0.05$.

Influence on the NO pathway

Blood pressure was measured in thiopental-anesthetized rats before and after intravenous administration of the investigated compounds (10 mg/kg b.w.) over a period of 15 minutes in all the groups. The left carotid artery was cannulated with polyethylene tubing filled with heparin solution in saline to facilitate pressure measurements made using Datamax apparatus (Columbus Instruments, Ohio). Subsequently, the animals were sacrificed. Blood was collected to heparinized tubes, centrifuged (286 x *g*, 10 min), and plasma was stored at – 80^{0}C. The livers, brains, hearts and kidneys were removed, washed in 0.9% NaCl, placed in liquid nitrogen and stored at -80^{0}C until needed for biochemical tests.

Preparation of tissue homogenates

The frozen tissues were weighed and homogenates were prepared by homogenization of 1 g of the tissue in 4 ml of 0.1 M phosphate buffer, pH 7.4 using IKA-ULTRA-TURRAX T8 homogenizer. Next, the homogenates were used for nitric oxide assay.

Determination of nitrate

NO was measured using Griess's method (Tsikas, 2007). The final products of NO *in vivo* are nitrite (NO_2^-) and nitrate (NO_3^-). Nitrite (NO) levels were measured in the homogenates or plasma. Briefly, 750 µl of redistilled water and 250 µl of tissue sample were placed in a water bath at 100°C for 15 min. After cooling, the samples were centrifuged at 10000 x g for 10 min. Nitrite determination: 75 µl of the supernatant and 75 µl of redistilled water were incubated for 30 min at room temperature. After this time, the absorbance was measured at 492 nm. Then, 50 µl of Griess reagents [sulfanilamide (1% solution in 2.5% H_2PO_3) and N-(1-naphtyl)-ethylenediamine (0.1% solution in 2.5% H_2PO_3)] were added and after 15 min the absorbance was measured at 492

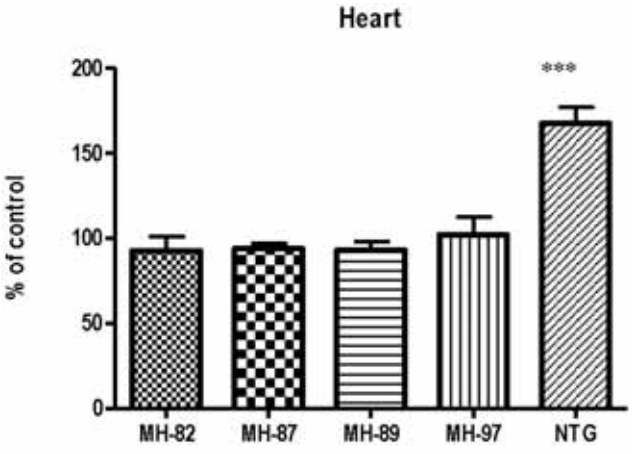

Fig. 1. Effects of the compounds and the reference compound NTG on the NO level in the rat heart.
Each value represents mean ± SEM. Statistical significance was evaluated using a one-way ANOVA test: ***p<0.001.

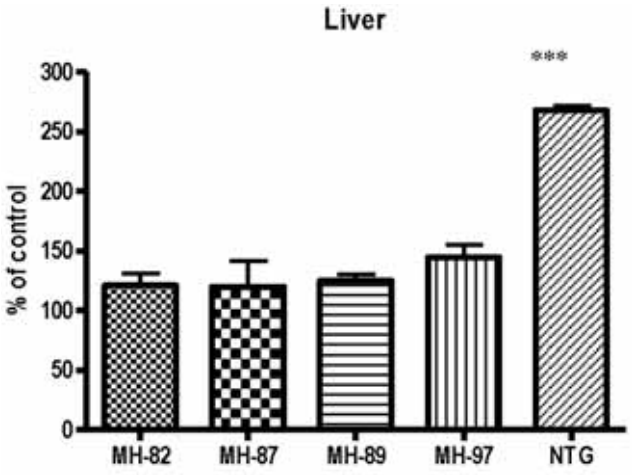

Fig. 2. Effects of the compounds and the reference compound NTG on the NO level in the rat liver.
Each value represents mean ± SEM. Statistical significance was evaluated using a one-way ANOVA test: ***p<0.001.

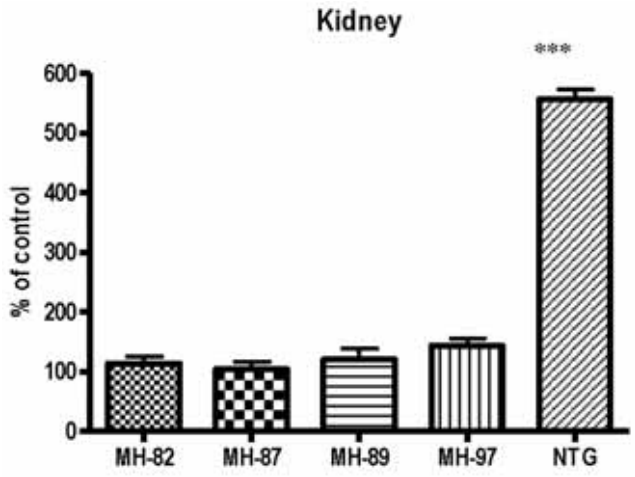

Fig. 3. Effects of the compounds and the reference compound NTG on the NO level in the rat kidney.
Each value represents mean ± SEM. Statistical significance was evaluated using a one-way ANOVA test: ***p<0.001.

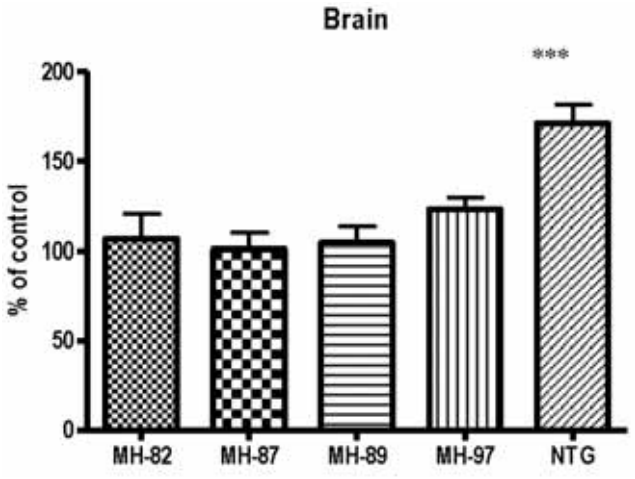

Fig. 4. Effects of the compounds and the reference compound NTG on the NO level in the rat brain.
Each value represents mean ± SEM. Statistical significance was evaluated using a one-way ANOVA test: ***p<0.001.

nm. The results were calculated from the standard curves obtained for sodium nitrite solutions (10 – 150 μM).

Determination of proteins

Proteins were measured using Lowry's method (Lowry et al., 1951). This method is based on the reaction of peptide bonds and aromatic amino acid residues of proteins with Folin-Ciocalteau reagent (a mixture of phosphotungstic acid and phosphomolybdenic acid) in an alkaline environment in the presence of cupric ions. Copper (II) ions bound to protein tyrosine and tryptophan residues reduce the above acids to oxides. Absorbance was measured at 500 nm. A 1% solution of bovine albumin was used to prepare a standard curve.

RESULTS

Nitroglycerine – the reference compound – significantly increased the NO levels in the tissues of the following organs: heart - about 68 % (control - 1.73±0.003 μM), liver - about 168 % (control - 1.10±0.007 μM), kidney - about 457 % (control - 0.89±0.011 μM), and brain - about 71 %. None of the tested compounds significantly increased the NO level in the heart, liver, kidney or brain tissues as compared with the control (100 %). These results indicate that the mechanism of hypotensive action of the examined new xanthone derivatives is not connected with the release of endogenous NO (Fig. 1, 2, 3, 4).

DISCUSSION

Pharmacological research on the new xanthone derivatives with potential biological properties has remained the area of our interest for the past several years and has been documented by a number of publications. In our previous research we examined a series of new aminoalcanolic derivatives of xanthone which exhibit high antiarrhythmic and/or hypotensive activity and high affinity for α/β_1-adrenoceptors (Marona et al., 2009). Additionally, we examined their two aminic analogues. Aminoalcanolic moieties are well-known structural elements of β-blockers. It is obviously known that the mechanism of action of nebivolol – the third-generation β-blocker – is also connected with the involvement of the NO pathway. Drugs that increase NO production can lead through this mechanism to vasodilation. It is an important mechanism of action because abnormalities in nitric oxide-mediated endothelial vasodilation are risk factors for atherosclerosis, hypertension or diabetes (Bacris et al., 2010; Kamp et al., 2010).

Xanthone derivatives can also act through the NO pathway. It has been shown that N-nitro-L-arginine methyl ester (L-NAME) – which is a NOS inhibitor and therefore leads to complete inhibition of NO production – inhibits endothelium-dependent action of xanthone derivatives (Wang et al., 2007). Nitric oxide is synthesized from L-arginine by NO synthase (NOS) in endothelial cells. It has also been found that endogenous inhibitors of NOS, such as asymmetric dimethylarginine (ADMA), which inhibit NO synthesis, are significantly increased in animals and patients with atherosclerosis. ADMA has been thought to be a key factor contributing to endothelial dysfunction. The protective effect of xanthones on the endothelial cells could be related to a decrease in ADMA concentration (Jiang et al., 2003). However, in our study the examined compounds did not increase the level of NO in tissues as compared with the control. In conclusion, the results of this study suggest that hypotensive activity of the tested xanthone derivatives is connected with high affinity for α/β_1-adrenoceptors but not with the release of endogenous nitric oxide.

ACKNOWLEDGMENTS

The authors would like to thank Mrs. Teresa Dobrut for her technical assistance.

REFERENCES

Bakris, G.L., J.N. Basile, T.D. Giles and A.A. Taylor. 2010. The role of nitric oxide in improving endothelial function and cardiovascular health: focus on nebivolol. *Am. J. Med*. 123, 2-8.

Dery, A.S., L.A. Hamilton and J.A. Starr. 2011. Nebivolol for the treatment of heart failure. *Am. J. Health Syst. Pharm*. 68: 879-86.

Jiang, D.J., Z. Dai and Y.J. Li. 2004. Pharmacological effects of xanthones as cardiovascular protective agents. *Cardiovasc. Drug Rev*. 22: 91-102.

Jiang, D.J., G.Y. Hu, J.L. Jiang, H.L. Xiang, H.W. Deng and Y.J. Li. 2003. Relationship between protective effect of xanthone on endothelial cells and endogenous nitric oxide synthase inhibitors. *Bioorg. Med. Chem*. 11: 5171-5177.

Hink, U., M. Oelze, P. Kolb, M. Bach schmid, M.H. Zou, A. Daiber, H. Mollnau, M. August, S. Baldus, N. Tsilimingas, U. Walter, V. Ullrich and T. Münzel. 2003. Role for peroxynitrite in the inhibition of prostacyclin syntase in nitrate tolerance. *J. Am. Coll. Cardiol*. 42: 1826-1834.

Kamp, O., M. Metra, S. Bugatti, L. Bettari, A. Dei Cas, N. Petrini and L. Dei Cas. 2010. Nebivolol: haemodynamic effects and clinical significance of combined beta-blockade and nitric oxide release. *Drugs* 70, 41-56.

Lin, K.W., S.C. Fang, C.F. Hung, B.J. Shieh, S.C. Yang, C.M. Teng and C.N. Lin. 2009. Synthesis, antiplatelet and vasorelaxing activities of xanthone derivatives. *Arch. Pharm. (Weinheim)*. 342: 19-26.

Lowry, O., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem*. 193: 265-275.

Marona, H., N. Szkaradek, A. Rapacz, B. Filipek, M. Dybała, A. Siwek, M. Cegła and E. Szneler. 2009. Preliminary evaluation of pharmacological properties of some xanthone derivatives. *Bioorg. Med. Chem*. 17:1345-52.

Marona, H., T. Librowski, M. Cegła, C. Erdoğan and N.O. Sahin. 2008 Antiarrhythmic and antihypertensive activity of some xanthone derivatives. *Acta Pol. Pharm*. 65: 383-90.

Marona, H., N. Szkaradek, M. Kubacka, M. Bednarski, B. Filipek, M. Cegla and E. Szneler. 2008a. Synthesis and evaluation of some xanthone derivatives for anti-arrhythmic, hypotensive properties and their affinity for adrenergic receptors. *Arch. Pharm. (Weinheim)*. 341: 90-8.

Napoli, C. and L.J. Ignarro. 2009. Nitric oxide and pathogenic mechanisms involved in the development of vascular diseases. *Arch. Pharm. Res*. 32: 1103-1108.

Santos, C.M., A.M. Silva, P. Filipe, R. Santus, L.K. Patterson, J.C. Mazière, J.A. Cavaleiro and P. Morlière. 2011. Structure-activity relationships in hydroxy-2,3-diarylxanthone antioxidants. Fast kinetics spectroscopy as a tool to evaluate the potential for antioxidant activity in biological systems. *Org. Biomol. Chem*. 9: 3965-3974.

Sy, G.Y., V. Bruban, P. Bousquet and J. Feldman. 2001. Nitric oxide and central antihypertensive drugs. One more difference between catecholamines and imidazolines. *Hypertension*. 37: 246-249.

Tsikas, D. 2007. Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: Appraisal of the Griess reaction in the l-arginine/nitric oxide area of research. *J. Chromatog*. B. 852: 51-70.

Wang, Y., J.G. Shi, M.Z. Wang, C.T. Che and J.H.K. Yeung. 2007. Mechanisms of the vasorelaxant effect of 1-hydroxy-2, 3, 5-trimethoxy-xanthone, isolated from a Tibetan herb, *Halenia elliptica*, on rat coronary artery. *Life Sci*. 81: 1016-1023.

Wang, L.W., J.J. Kang, I.J. Chen, C.M. Teng and C.N. Lin. 2002. Antihypertensive and vasorelaxing activities of synthetic xanthone derivatives. *Bioorg. Med. Chem*. 10: 567-72.