Acta Veterinaria (Beograd), Vol. 63, No. 4, 363-371, 2013.

DOI: 10.2298/AVB1304363I UDC: 615.222: 616-007.281+591.413: 599.1.3./.8.4.

THE NOVEL ORTHO-CHLORO DERIVATE PROPAFENONE INDUCED RELAXATION IN ISOLATED RAT AORTA

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(Received 12th March, 2013)

The information on the inhibitory effect of propafenone in vascular smooth muscle is sparse. Propafenone acts through blockage of voltage-dependent cardiac Na^+ channels, L-type Ca^{2+} channels, voltage-sensitive K^+ (Kv) channels, as well as β-adrenergic receptors in the heart. The introduction of different chemical groups in the benzyl moiety of propafenone influences pharmacological properties of newly developed derivate of propafenone. Here we investigated the effect of new ortho-chloro derivate of propafenone (5OCl) on the vascular tone of precontracted rat aorta. 5OCl produced endothelium-independent relaxation of rat aorta. In order to test the involvement of different ion channels in 5OCl mechanism of action, antagonist of Na^+, lidocaine, K_V channels, 4-aminopyiridine (4-AP) and L-type Ca^{2+} channels, nifedipine were used. All tested antagonists of ion channels did not influence the relaxation of rat aorta induced by high a concentration of 5OCl (≥10 μM), but antagonized the relaxation induced by low concentrations of this propafenone derivate. Thus, 5OCl derivate has comparable potency and efficacy as propafenone. According to its interaction with lidocaine, 4-AP and nifedipine it seems that 5OCl partly shares the mechanism of action with propafenone. The mechanism of vasodilatation induced by high micromolar concentration of 5OCl is not defined and further investigations are necessary.

Key words: ion channels, ortho-chloro derivate of propafenone, rat aorta, relaxation

INTRODUCTION

Propafenone is a class Ic antiarrhythmic drug used to maintain sinus rhythm in patients with supraventricular tachycardia, including atrial fibrillation (Goodman and Gilman, 2003). Propafenone acts through blockage of voltage-dependent

cardiac Na^+ channels (Malfatto *et al*., 1988; Valenzuela *et al*., 1988). However, may also inhibit the L-type Ca^{2+} channels, K^+ channels, as well as β-adrenergic receptors in the heart (Malfatto *et al*., 1988; Bryson *et al*., 1993; Franqueza *et al*., 1998). The information on the inhibitory effect of propafenone in vascular smooth muscle is sparse.

Perez-Vizcaino *et al*. (1994) have shown that propafenone inhibited contractions of rat aorta and porcine coronary artery which could be attributed to reduced Ca^{2+} entry. A decrease in systemic vascular resistance may reduce ventricular afterload, while a decrease in coronary vascular resistance might increase coronary blood flow. Thus, it is possible that in some patients these vasodilatator actions of propafenone may improve the cardiac performance, overriding its cardiodepressant effect. A possible role for Na^+ channel inhibition in the observed inhibitory effect of propafenone was excluded. Propafenone decreased the contraction of rat aorta induced by high KCl due to its L-type Ca^{2+} channel blocking properties (Fernandez del Pozo *et al*., 1996). In contrast to this, in the same blood vessel, propafenone inhibited the vasodilatation induced by K^+ channel opener, levcromakalim in a non-competitive manner. The concentration at which this effect was observed within the therapeutic range and similar to the one reported to inhibit ATP-sensitive K^+ (K_{ATP}) channels (Cogolludo *et al*., 1998). Later, the same authors have shown that propafenone (10 μM) inhibited voltage-sensitive K^+ (K_V), big calcium-sensitive K^+ (BK_{Ca}) and K_{ATP} channels in the isolated smooth muscle cell from rat portal veins (Cogolludo *et al.*, 2001). The inhibition of K^+ channels is expected to produce membrane depolarization, activation of L-type Ca^{2+} channels and vasoconstriction (Nelson and Quayle, 1995). However, the intravenous administration of propafenone produced a transitient and slight fall in systolic blood pressure (Feld *et al*., 1987). It is possible that at certain concentrations the direct effect of propafenone on vascular K^+ channels may be counteracted by the blockage of L-type Ca^{2+} channels (Cogolludo *et al*., 2001).

It is well known that the presence of different chemical groups in drug's molecules influenced its potency, selectivity and mechanism of action. The computer-based analysis of substructures of various response modifiers suggested secondary amino groups, ethers, carbonyl groups and benzyl groups in the propafenone as possible pharmacophoric substructures (Chiba *et al*., 1995). Thus, we have shown that propafenone and its derivates which were syntethysed by substitution in the benzyl moiety with -F, -CH3 or CF3 groups on the *ortho* or *para* position produced vasodilatation of rat aorta. Introduction of 5-ortho-CF3 and 5-para-CH3 group in the benzyl moiety of propafenone molecule changed its potency and Kv and L-type Ca^{2+} channels are involved in their mechanism of action. The introduction of other tested groups in the benzyl moiety did not influence pharmacological properties of derivates of propafenone in relation to propafenone. Analogs of propafenone were synthesized with modifications in the benzyl moiety designed on the basis of the pre structure property relationship (SPR) and pharmacophoric studies (Ivkovic *et al*., 2012).

Here we investigated the effect of new *ortho*-chloro derivate of propafenone (5OCl) on the vascular tone of rat aorta (Fig. 1). According to great efficacy of

propafenon in therapy of arrhythmia, the development of new structurally related propafenone derivates with better clinical profile may be useful in future.

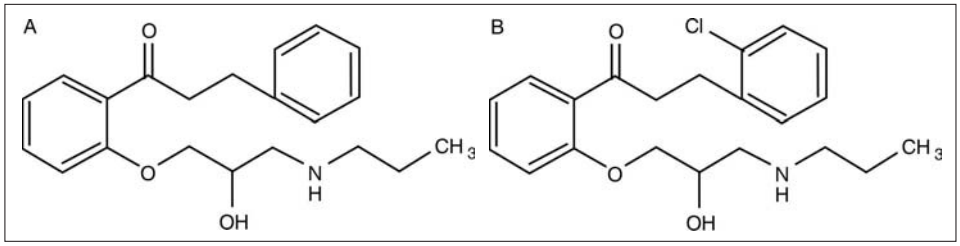

Figure 1. Chemical structures of (A) propafenone and (B) its *ortho* chloro derivate 5OCl

MATERIAL AND METHODS

Assessment of vascular function

Male Wistar rats obtained from the animal facilities of the University of Belgrade of Medicine faculty were used in all experimental procedures. The study was approved by the Ethical Committee of Faculty of Medicine, University of Belgrade. The studies reported in this work have been carried out in accordance with the Guide for the Care and Use of Laboratory Animals are adopted and promulgated by the United States National Institutes of Health.

Vascular rings were prepared from the aorta of male Wistar rats, body weight of 250 – 300 g. The aorta segments were dissected free from connective tissue. They were cut into rings (3 mm) and mounted between two stainless-steel triangles in an organ bath containing 10 mL Krebs-Ringer-bicarbonate solution (mmol/L: NaCl 120, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, T = 37°C, pH = 7.4), aerated with 95% O_2 and 5% CO_2. One of the triangles has been attached to a displacement unit allowing fine adjustment of tension and the other was connected to an isometric transducer (K30; Hugo Sachs, Freiburg, Germany). The preparations were allowed to equilibrate for 30 min. We have examined the effects of 5OCl onto the rings with or without endothelium. After the equilibration period, the presence of functional endothelium was assessed. Rings were precontracted with phenylephrine (10 μM) and acetylcholine (20 μM) was added into the organ bath. In the some experiments endothelium was removed mechanically. The resting tension was 2 g. The vascular rings were allowed a further 30 min to equilibrate before being contracted with phenylephrine (10 μM).

Contraction of the rat aorta evoked by phenylephrine

Concentration-response curves were obtained by the cumulative addition of 5OCl to ring segments contracted to a stable plateau by adding phenylephrine. Increasing concentrations of 5OCl has been added only after the previous concentration had produced equilibrium response. Therefore, the following protocol was used: 1) contraction to phenylephrine and concentration - response

curve to 5OCl followed by three washes, addition of ion channel blockers (3 mM 4-aminopyridine or 1 μM nifedipine or 3 mM lidocaine) and 20 min equilibration period; 2) contraction to phenylephrine and the concentration-response curve to 5OCl.

Statistical analyses

The results are expressed as the means ± standard error (SEM); n refers to the number of experiments. The concentration of 5OCl producing 50% of its own maximum response (EC_{50}) was determined for each curve by using a non-linear least square fitting procedure of the individual experimental data. The pD_2 value was calculated from -log EC_{50}. Responses are expressed as a percentage of the maximum possible relaxation, i.e., the return of tension to the pre-phenylephrine level (Emax). All calculations were done by using the computer program Graph Pad Prism (Graph Pad Software Inc., San Diego, U.S.A.). Statistical difference between means was determined by Student's t-test and a p value <0.05 was considered statistically significant.

Drugs

For chemical synthesis of 5OCl derivate the following compounds are used: 2-hydroxyacetophenone, 2-chlorobenzaldehyde, epichlorhydrine, propylamine, and hydrochloride acid (Sigma-Aldrich Inc., St. Louis, MO, USA).

In pharmacological experiments the following drugs were used: phenylephrine, acetylcholine, 4-aminopyridine (4-AP), nifedipine, lidocaine (Sigma-Aldrich Inc., St. Louis, MO, USA). 5OCl was dissolved in distilled water. Nifedipine was dissolved in 70% v/v ethanol with further dilution in distilled water before use. Working concentrations of ethanol in the bath were <0.01% (v/v). Previous experiments showed that the solvents used had no effects on preparations at the applied concentrations. All drugs were added directly to the bath in a volume of 100 μL and the concentrations given are the calculated final concentrations in the bath solution. The experiments with nifedipine were performed in a dark room.

RESULTS

Synthesis of phenylpropiophenone derivative

Synthesis of the 5OCl compound was carried out according to the general method first reported by Ivkovic *et al*., (2009) and its structure has been verified by NMR, MS and MS spectroscopy. The tested compound is derivative of phenylpropiophenone (as well as propafenone) with chloro substituent on the *ortho* position of the benzyl moiety (Fig. 1). Because of the high structural similarity between newly synthesized compound and propafenone, this compound may be considered derivatives of propafenone.

Relaxant effects of 5OCl on the isolated rat aorta precontracted by phenylephrine

5OCl (1 - 100 μM) induced a concentration dependent relaxation of rings with endothelium and without endothelium with EC_{50} values of 13.51 ± 0.09 μM (E_{max} = 86.1 ± 3.9%, n = 6) and 14.20 ± 0.10 μM (E_{max} = 90.1 ± 2.8%, n = 6)

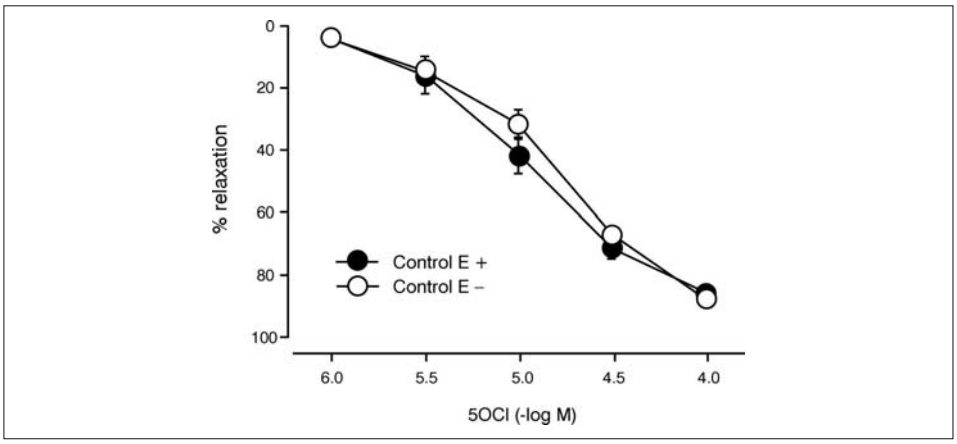

Figure 2. Cumulative concentration-response curves to 5OCl in the rat aorta with endothelium (black circle) and without endothelium (white circle) precontracted with phenylephrine (10 ìM). Responses are expressed as a percentage of the maximum possible relaxation (i.e., return to baseline tension). Each point represents the mean ± S.E.M. (n = 12)

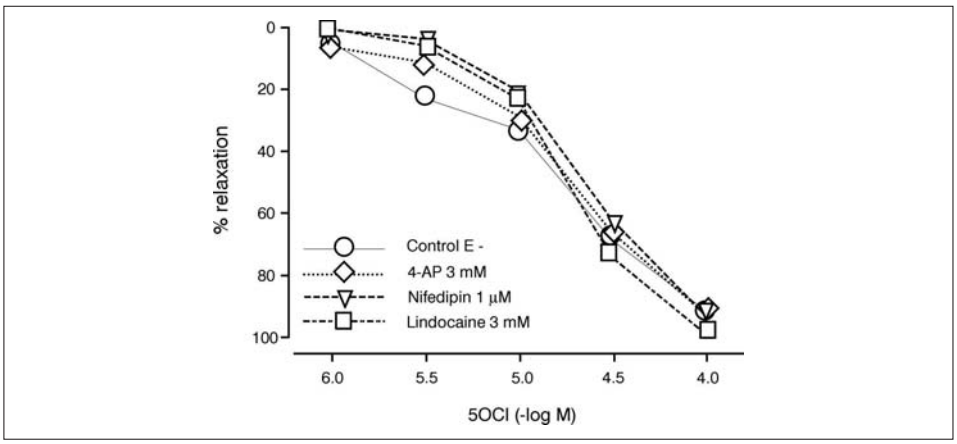

Figure 3. Antagonism of the relaxation effects of 5OCl by 4-AP, nifedipine and lidocaine on tension development in the rat aorta ring. Concentration-response curves for 5OCl in the absence (open circle) and presence of 4-AP (3 mM, open diamond), nifedipine (1 μM, open triangle) and lidocaine (3 mM, open square). Each point represents the mean ±S.E.M. (n = 4-7)

respectively ($p>0.05$, Fig. 2). Regarded EC_{50} values, 4-AP (3 mM, n = 7) did not modify the 5OCl induced-relaxation of the rat aorta (EC_{50} = 16.60 ± 0.1 μM, E_{max}= 91.1 ± 3.5 %, $p> 0.05$). Further, nifedipine (1 μM, n = 4) did not modify the relaxation of the rat aorta induced by 5OCl (EC_{50} = 20.25 ± 0.30 μM, Emax = 92.0 ± 4.5 %, $p>0.05$). Lidocaine (3 mM, n = 6) did not modify the 5OCl induced relaxation of the aorta rings (EC_{50} = 13.92 ± 0.11 μM, Emax = 97.2 ± 2.5 %, $p<0.05$). However, 4-AP, nifedipine and lidocaine significantly inhibited the relaxation elicited with concentration of 5OCl less than 10 μM (EC_{25} without antagonist was 5.34 ± 0.11 μM; in the presence of 4-AP, nifedipine and lidocaine EC_{25} were 5.07 ± 0.14 μM, 4.93 ± 0.18 μM and 4.99 ± 0.09 μM, respectively, $p<0.05$, Fig. 3)

4 - AP (3 mM), nifedipine (1 μM) and lidocaine (3 mM), did not affect the basal tension of aorta nor the contraction induced by phenylephrine (data not shown, n = 3 - 7).

DISCUSSION

5OCl induced endothelium-independent relaxation of rat aorta. This result is consistent with previous findings for propafenone-induced dilatation of rat aorta and portal vein (Carron *et al*., 1991; Cogolludo *et al*., 1998). Also, we have shown previously that five newly developed phenylpropiophenone derivate produced endothelium-independent relaxation of rat aorta (Ivkovic *et al*., 2012), as well. The potency of 5OCl (4.85) calculated from the pD_2 value from the concentration-response curves was significantly lower than the potency of propafenone (5.03) and its derivates, ortho-CF_3 (5.12) and ortho-CH_3 (4.96) (Ivkovic *et al*., 2012). It is obvious that the presence of different chemical groups in the molecule of propafenone influences its potency.

In order to test the involvement of different ion channels in 5OCl mechanism of action, antagonist of Na^+, K_V and L-type Ca^{2+} channels were used.

To analyze the contribution of Na^+ channels to the 5OCl-induced relaxation of the rat aorta, lidocaine was used. Lidocaine is the local anesthetic that blocks $Na_v1.5$ channels and has been used therapeutically to manage cardiac arrithmias (Glaaser and Clancy, 2006). Our data suggest the involvement of lidocaine-sensitive channels in the relaxation of the rat aorta by 5OCl (if it is used in concentration lower than 10 μM). The rank order of concentration ratio (CR) value in presence of lidocaine was 0.98. Lidocaine produced a stronger inhibition of the propafenone effect in the same experimental model (CR = 1.37; Ivkovic *et al*., 2012). Lidocaine did not block the effect of higher concentrations of 5OCl (≥10 μM). It seems that 5OCl has a complex mechanism of action.

To analyze the contribution of K^+ channels to the 5OCl-induced relaxation of the precontracted aorta's ring, we used 4-AP, a blocker of K^+ channels (Wulff and Zhorov, 2008). Used in low milimolar concentratons, 4-AP achieved some selectivity for Kv channels (O'Rourke, 1996). In this study the CR values of 5OCl and 4-AP (1.17) was comparable with CR values obtained for ortho-CH_3 (1.17) derivate of propafenone and 4-AP (Ivkovic *et al*., 2012). In contrast to the results

presented here, it has been shown that propafenone blocks the open state of K^+ channel (Delpon *et al*., 1995, Duan *et al*., 1993, Arias *et al*., 2003). The effect of propafenone depends on whether the membrane is in a resting potential or in a state of depolarization (Lemennas - Gruber *et al*., 1997). Our finding supports a relavant participation of Kv channels in the relaxation of the aorta ring produced by 5OCl if it is used in concentrations ≤10 μM. As propafenone, 5OCl in concentrations >10 μM acts through Kv channel–independent mechanism of action (Arias *et al*., 2003; Ivkovic *et al*., 2012).

In order to analyze the contribution of the L-type Ca^{2+} channels to the 5OCl-induced relaxation of the rat aorta, we used nifedipine. Nifedipine (1 μM) caused significant antagonism of the effect of = 10 μM 5OCl suggesting the involvement of the nifedipine-sensitive Ca^{2+} channels. In rat isolated aortic strips and portal veins nifedipine inhibited the propafenone effect with similar potency (Carron *et al*., 1991; Perez-Vizcaino *et al*., 1994; Ivkovic *et al*., 2012). However, we have shown that nifedipine inhibited more potently the effect of other propafenone derivate, i.e. *ortho*-CF_3 (Ivkovic *et al*., 2012). As propafenone, high micromolar concentrations of 5OCl have ion a channel-independent mechanism of action (Ivkovic *et al*., 2012). It has been shown previously that propafenone inhibited Ca^{2+} release from intracellular stores in the rat aorta (Carron *et al*., 1991).

The introduction of Cl group on the *ortho* position in the bezyl moiety of propafenone changed its effect on the precontracted rat aorta. 5OCl derivate has comparable potency and efficacy as propafenone. According to its interaction with lidocaine, 4-AP and nifedipine it seems that 5OCl partly shares the mechanism of action with propafenone. The mechanism of vasodilatation induced by high micromolar concentration of 5OCl is not defined and further investigations are necessary. But we must be cautious in extrapolating their results to *in vivo* conditions.

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RELAKSACIJA AORTE PACOVA INDUKOVANA NOVOSINTETISANIM ORTO-HLORNIM DERIVATOM PROPAFENONA

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SADRŽAJ

Informacije o efektima propafenona na vaskularne glatke mišiće su oskudne. Propafenon blokira voltažno-zavisne Na^+ kanale, Ca^{2+} kanale L-tipa, voltažno-senzitivne K^+ (Kv) kanale i β-adrenergičke receptore u srcu. Uvođenje različitih hemijskih grupa u benzilni deo molekula propafenona utiče na promenu njegovih farmakoloških osobina. U ovoj studiji je ispitivan uticaj novog orto-hloro derivata (5OCl) propafenona na vaskularni tonus prekontrahovane aorte pacova. Orto hlorni derivat (5OCl) je izazvao endotel-nezavisnu relaksaciju aortnih prstenova. Da bi se ispitala uloga različitih jonskih kanala u ovoj relaksaciji, korišćeni su lidokain, (antagonist Na^+ kanala), 4-aminopiridin (antagonist Kv kanala) i nifedipin (antagonist Ca^{2+} kanala L-tipa). Testirani antagonisti jonskih kanala nisu uticali na relaksaciju aorte pacova izazvanu visokom koncentracijom 5OCl ($\geq$10 μM), ali su zato antagonizovali relaksaciju aorte koncentracijama 5OCl koje su bile manje od 10 μM. Prema tome, 5OCl derivat ima sličnu jačinu i efikasnost kao propafenon. Prema njegovoj interakciji sa lidokainom, 4-AP i nifedipinom može se reći da je mehanizam dejstva 5OCl sličan propafenonu. Mehanizam vazodilatacije 5OCl derivata u koncentracijama većim od 10 μM nije definisan i za to su potrebna dalja istraživanja.