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Propranolol-induced relaxation in the rat basilar artery

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ABSTRACT

Propranolol is a non-selective beta-adrenergic receptor blocker used in the treatment of cardiovascular diseases and migraine prophylaxis. Although it has been shown that propranolol dilates the peripheral arteries of rat, its action in the central nervous system vasculature has not been investigated. In this study, the effects of propranolol in rat basilar artery were investigated. Basilar arteries from male Wistar rats were examined in a myograph system. The relaxant effects of propranolol, pindolol, atenolol, pizotifen and methysergide were examined in basilar arteries precontracted by serotonin or PGF2α. Only propranolol and pizotifen induced vasorelaxations; the pD2 values were 5.23 ± 0.13 and 5.94 ± 0.03; respectively. The vasorelaxation induced by propranolol and pizotifen was not affected by endothelium or the presence of L-NOARG and/or indomethacin. The calcium channel blocking activity of propranolol and pizotifen was compared with that of nifedipine in a calcium free solution with high K^+ (60 mM) concentration. These drugs shifted the concentration–response curves of calcium induced contractions with pA2 values of 5.45 ± 0.04; 7.14 ± 0.09; and 9.22 ± 0.06 respectively. The P2Y receptor agonist UTP was used to induce sustained and stable contractions in basilar artery segments. Nifedipine caused a marked, but an incomplete relaxation. Cyclopiazonic acid, an inhibitor of sarcoplasmic reticulum calcium channels, but not propranolol or pizotifen abolished the remaining tonus after partial relaxations obtained with nifedipine.
These results suggest that propranolol causes vasorelaxation by blocking the L-type voltage-gated calcium channels in the rat basilar artery.

1. Introduction

Propranolol is a beta adrenergic receptor antagonist (Black et al., 1965)—a pure antagonist that is highly lipophilic (Reiter, 2004). Propranolol has antagonistic effects on beta-1, beta-2, and beta-3 adrenergic receptors (Baker, 2005). It has a membrane stabilizing local anesthetic effect, but no partial agonistic action (Reiter, 2004). Propranolol is used for the treatment of many cardiovascular diseases, including hypertension, angina, chronic heart failure, and arrhythmias (Kaplan, 1997; Hjalmarson, 1997; Ellison and Gandhi, 2005; Murray et al., 1990). Propranolol is also used for the treatment of glaucoma (Alward, 1998) and as a prophylactic for migraine headache (Linde, 2004).

The therapeutic mechanism of action of beta-receptor antagonists has yet to be fully discerned. It is known that non-selective beta adrenergic receptor blockade in arteries increases peripheral resistance as a result of the antagonism of beta-2 receptors (Reiter, 2004). Yet, following long-term use of beta-blockers, total peripheral resistance returns to normal values (Reiter, 2004). More recently, an increase in nitric oxide (NO) synthesis has been proposed as a possible mechanism of the antihypertensive effect of beta-blockers (Kurosaki et al., 2000; Broeders et al., 2000). It was also reported that propranolol causes vasorelaxation in rat mesenteric artery via the release of NO and the blockade of calcium channels (Priviero et al., 2006). These effects are independent of beta-adrenergic receptor blockade (Priviero et al., 2006). Inhibitory effects on the thromboxane system by the kidneys also seem to contribute to the therapeutic effects of beta-blockers (Hirawa et al., 1991).

The maintenance of cerebral blood flow is critically important for sustaining life. Sudden changes in cerebral arterial blood flow result in pathological conditions, such as ischemia (vasoconstriction) and migraine (vasodilatation). Cerebrovascular pathologies result in neurological disorders and life-threatening complications. The rat basilar artery is a cerebral vessel (approximately 150 μm in diameter) that plays an important role in the regulation of cerebrovascular blood flow and supplies blood to important regions of the brain.

Migraine headache episodes are related with cerebral blood flow (Villalon et al., 2003). The release of vasodilator substances from the complex trigeminovascular innervations around arteries plays a role in the migraine pathogenesis (Villalon et al., 2003). Many drugs of various classes have been used for the treatment and prevention of migraine headache. Propranolol has been used for migraine prophylaxis effectively (Linde, 2004). Some calcium channel blockers such as flunarizine are being used for migraine treatment; however, nifedipine–a calcium channel blocking drug–causes vasodilation that results in the exacerbation of migraine symptoms.

The vasorelaxant effect of propranolol was observed in the rat aorta, and mesenteric and coronary arteries (Priviero et al., 2006; Sakanashi and Takeo, 1983); however, the effect of propranolol on the rat basilar artery has not been investigated. Observation of the vasorelaxant effect of propranolol on the basilar artery would be quite valuable, as there is a

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contradiction between the drug's mechanism of anti-migraine effect and its vasorelaxing effect in cerebral arteries. We hypothesized that the vasorelaxant effect of propranolol in rat basilar artery is independent of beta-adrenergic receptor blockade. As such, the present study aimed to examine the functional effects of propranolol in the rat basilar artery.

2. Material and methods

2.1. Animals

Male Wistar rats weighing 220–350 g were used for the study. The use of experimental animals and the study protocol were approved by the Hacettepe University Animal Care Committee (2008/36).

2.2. Preparation of basilar arteries and isometric tension recording

The rats were anesthetized using diethyl ether. Following decapitation, the basilar artery was isolated and dissected on a Petri dish filled with ice-cold physiological salt solution (PSS) composed (in mmol L^{-1}) of the following; NaCl: 118; KCl: 4.6; $NaHCO_3$: 25; $MgSO_4$: 1.2; KH_2PO_4: 1.2; $CaCl_2$: 1.2; glucose: 10; and EDTA: 0.025. Basilar artery rings were isolated from the surrounding tissue and cut into segments of approximately 2 mm. Isolated artery rings were threaded onto 2 stainless steel wires (40 μm in diameter) and mounted in a wire myograph (myograph 610M, Danish Myo-Technology, Aarhus, Denmark). The wire myograph bath contained 5 mL of PSS, which was continuously aerated with a gas mixture (95% O_2 and 5% CO_2) in order to maintain the pH at 7.4. After mounting, the vessels were allowed to equilibrate for 30 min at 37 °C. Isometric responses were recorded as mN using a PowerLab/4SP computer system (AD Instruments, CO, USA). The arterial rings were normalized using a normalization module program (Danish Myo-Technology, Aarhus, Denmark) via stepwise stretching (Mulvany and Halpern, 1977).

Following the normalization procedure and 30-min equilibration period, the basilar arteries were stimulated with isotonic high-K^+ PSS (60 mM K^+). The concentration of NaCl was rearranged to obtain an isotonic solution and the final concentrations (in mM) in the isotonic high K^+ PSS (60 mM K^+) solution were as follows; NaCl: 62.6; KCl: 60; $NaHCO_3$: 25; $MgSO_4$: 1.2; KH_2PO_4: 1.2; $CaCl_2$: 1.2; glucose: 10; and EDTA: 0.025. The endothelium was removed mechanically from some rings by rubbing the intimal surface of the vessels with steel wires (40 μm in diameter). Acetylcholine (1 μM) was applied on serotonin (0.3 μM)-precontracted arteries to test whether the endothelium was intact or not. In endothelium intact arteries acetylcholine caused approximately 60% relaxation. The absence of endothelium was confirmed by the loss of relaxation (0–5%) to acetylcholine at the beginning of the experiments.

3. Experimental protocol

3.1. Vasorelaxation induced by propranolol

After 5 min of stimulation with high-K^+ PSS, segments were washed with normal PSS and equilibrated for 30 min. Precontraction was achieved with serotonin (0.3 μM) or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 3 μM). Following a stabilization period, propranolol was added cumulatively (10 nM–30 μM) to the organ bath to investigate the vasorelaxant effect on endothelium-intact and endothelium-denuded basilar arteries. Responses to propranolol were also observed in the presence of indomethacin (10 μM) and Nω-nitro-L-arginine (L-NOARG; 100 μM) alone, and indomethacin (10 μM) + L-NOARG (100 μM). Papaverine (PAP, 0.1 mM) was added at the end of each experiment to assess the vascular relaxing capacity.

In another group of experiments, pindolol (10 nM–30 μM; a non-selective beta-receptor antagonist) and atenolol (10 nM–30 μM; a beta-1 selective antagonist) were administered cumulatively to compare their effects to those of propranolol. As propranolol has a local anesthetic effect, we also tested the local anesthetic drug lidocaine (10 nM–30 μM) in another group of experiments to determine if it has an effect similar to that of propranolol. Additionally, the effects of pizotifen (10 nM–30 μM) and methysergide (10 nM–30 μM), which are other drugs used for chronic prophylaxis of migraine, were compared to those of propranolol. PAP, 0.1 mM, was added at the end of each experiment to assess the vascular relaxing capacity.

3.2. Calcium channel blocking effect

Following the normalization period, PSS was substituted with high-K^+ PSS that did not contain Ca^{2+}. Arteries were incubated with serotonin (0.5 μM) and Ca^{2+}-ATPase inhibitor cyclopiazonic acid (CPA, 20 μM) to deplete the intracellular calcium stores and to prevent Ca^{2+} uptake by the sarcoplasmic reticulum, respectively (Priviero et al., 2006; Lagaud et al., 1999). Calcium chloride ($CaCl_2$; 0.1 μM–3 mM)-induced contractions were observed and recorded in the absence and in the presence of different concentrations of propranolol. Methysergide and pizotifen were administered to determine if they have calcium-antagonizing effects. Nifedipine, a potent L-type Ca^{2+} channel blocker, was used to compare calcium channel blocking effects. To investigate the effects of the drugs on intracellular calcium stores and Na^+/Ca^{2+} exchangers, following an equilibration period, uridine 5′-triphosphate trisodium salt dihydrate (UTP; 0.1 mM) was used to maintain a stable tonic contraction (Syyong et al., 2009). Nifedipine (10 μM) was added to the UTP contraction to eliminate the role of voltage gated L-type calcium channels; residual tonic contraction remained following the addition of nifedipine. The remaining nifedipine-insensitive tonic contraction was due to intracellular calcium dynamics and Na^+/Ca^{2+} exchangers. Because only propranolol and pizotifen exhibited calcium-antagonizing effects, they were tested after the administration of nifedipine on the tonic contraction elicited by UTP to investigate their effects on this remaining tonus.

3.3. Drugs

The following drugs were used in the study; acetylcholine, cyclopiazonic acid, $PGF_{2\alpha}$, UTP, lidocaine, L-NOARG, serotonin (Sigma-Aldrich, St. Louis, MO, USA), propranolol, atenolol (Biofarma, Istanbul, Turkey), nifedipine (Bayer, Leverkusen, Germany), methysergide maleate, pindolol, pizotifen malate (Sandoz, Basel, Switzerland), and indomethacin (Knoll Chemicals, Germany). All drugs were dissolved in distilled water, except for nifedipine, pindolol, indomethacin, L-NOARG, and pizotifen. Nifedipine was dissolved in ethanol, and pindolol and pizotifen were dissolved in DMSO. L-NOARG was dissolved in 0.1 N HCl and indometacin was dissolved in 6.85 mM Na_2CO_3. Further dilutions were prepared in distilled water.

3.4. Statistical analysis

Vascular responses were recorded as mN mm^{-1} and relaxations are shown as a percentage of precontraction due to serotonin, $PGF_{2\alpha}$, or UTP. $CaCl_2$-induced contractions were calculated as a percentage of 61.2 mM K^+ contractions. Differences between curves were analyzed using two-way repeated-measures ANOVA. pD_2 (the negative logarithm of the mean concentration of the agonist that caused half-maximum contraction, EC_{50}) and pA_2 (the negative logarithm of the mean antagonist concentration that increased the agonist concentration to double that induced 50% of the maximum effect, E_{max}) were assessed using non-linear curve fit analysis of the semi-logarithm concentration–response curve via GraphPad Prism v.5.0 (La Jolla, CA, USA). The level of statistical significance was set at $P<0.05$.