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Ouabain treatment changes the role of endothelial factors in rat resistance arteries

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ABSTRACT

This study investigates the participation of the endothelial factors in the α-adrenoceptor contractile responses in mesenteric resistance arteries from 15 days ouabain-treated (25 μg/kg/day) and untreated rats. Ouabain treatment increased blood pressure and heart rate without changing the contractile response to phenylephrine (3 nM–30 μM). Endothelium removal or N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM), increased the responses to phenylephrine. The endothelial modulation was similar in both rat groups, but the L-NAME effects were bigger in arteries from ouabain-treated rats. However, the endothelial NOS expression and the relaxation to acetylcholine (0.1 nM–10 μM) remained unaltered after ouabain treatment. The coincubation with L-NAME and indomethacin (100 μM) leftward shifted the concentration–response curves to phenylephrine in arteries from untreated rats similarly to the displacement after incubation only with L-NAME. However, in mesenteric arteries from treated rats, the co-incubation with indomethacin and L-NAME did not alter the response to phenylephrine. The addition of the inhibitor of calcium activated potassium channels tetraethylammonium (2 mM) further leftward shifted the phenylephrine curves only in arteries from untreated rats. Cyclooxygenase-2 (COX-2) expression was greater in vessels from ouabain-treated rats. In conclusion, the chronic ouabain treatment for 15 days modified the participation of endothelial factors in response to phenylephrine in mesenteric resistance arteries, by increasing the release of NO and prostanoids and impairment the endothelium-derived hyperpolarizing factor (EDHF) release. This was accompanied by an increased COX-2 expression. Although this balance avoids changes in the phenylephrine concentration–response curves, these vascular changes might contribute to maintain the ouabain-induced hypertension.

1. Introduction

Several reports have demonstrated the presence of nanomolar concentrations of a digitalis compound in the plasma of hypertensive humans and rats (Hamlyn et al., 1982; Blaustein, 1993; Hamlyn et al., 1996). This endogenous compound was characterized as ouabain or an isomer of ouabain (Mathews et al., 1991) and its chronic administration to rats increases arterial blood pressure (Yuan et al., 1993; Manunta et al., 1994; Huang et al., 1994; Kimura, et al., 2000; Manunta et al., 2001; Rossoni et al., 2002a,b). Central and peripheral mechanisms seem to be involved in the hypertension induced by chronic ouabain administration. The central mechanism is associated with the increase of sympathetic tone and impairment of the baroreflex (Huang and Leenen, 1999). Among the peripheral mechanisms, the inhibition of the sodium pump is included promoting the increase of intracellular sodium concentration that reduces or reverses the activity of the Na^+/Ca^{2+} exchanger (Marín and Redondo, 1999).

When acutely administered ouabain increases vascular reactivity (Vassallo et al., 1997; Padilha et al., 2004) and increases or reduces the Na^+K^+ATPase activity, depending on its concentration (Rossoni et al., 1999; Padilha et al., 2004). However, after chronic ouabain administration a reduction of vascular reactivity to phenylephrine together with an increased activity and expression of the Na^+K^+ATPase occurs (Rossoni et al., 2002a). In addition, increased endothelial nitric oxide synthase (eNOS) expression and endothelial NO modulation of vasoconstrictor responses was also observed (Rossoni et al., 2002b). However, the vascular effects of the chronic ouabain treatment seem to be dependent on the studied vessel. Thus, in conductance arteries, chronic ouabain treatment increases NO production and impairs prostanoid actions reducing the vasoconstriction produced by α-adrenergic agents (Rossoni et al., 2002b; Xavier et al., 2004b). Meanwhile, in mesenteric

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resistance arteries although presenting an enhancement of NO production, there is a reduction of the EDHF action and then, the vascular reactivity to norepinephrine remains unchanged (Xavier et al., 2004b). All those vascular effects were studied after five weeks of ouabain administration but it is described that the increase in arterial blood pressure is already present after two weeks of ouabain administration (Rossoni et al., 2002a,b; Xavier et al., 2004a,b). Besides, it is well known that resistance arteries play the major role in the regulation of the peripheral resistance and therefore to the blood pressure. The aim of the present study was to analyze the effect of hypertension induced by 15 days ouabain treatment on the endothelial modulation of vascular responses induced by α-adrenoceptor activation in resistance arteries.

2. Materials and methods

2.1. Animals

Male 10-week old Wistar rats obtained from colonies maintained at the Animal Quarters of the *Facultad de Medicina* of the *Universidad Autónoma de Madrid* were used. During treatment, rats were housed at constant room temperature, humidity and light cycles (12-h light/dark), they had free access to tap water and were fed with standard rat chow *ad libitum*. Care and use of laboratory animals and all experiments were conducted in compliance with the guidelines for biomedical research as stated by the Spanish and European laws (RD 223/88 MAPA and 609/86).

Rats were divided in two groups: Control Rats ($N=10$) received saline (*s.c.*); Ouabain Rats ($N=10$), received 25 μg/kg/day ouabain diluted in soy oil (*s.c.*), for 15 days. At the end of the treatment, rats were anesthetized with urethane (1.2 g/kg, *i.p.*) and a polyethylene catheter (PE50), filled with heparinized saline (50 U/ml), was introduced into the carotid artery. The systolic and diastolic blood pressures were measured during 30 min with a pressure transducer (model 1050BP, UFI, Inc., Morro Bay, CA) and recorded using an interface and software for computer data acquisition (model MP100A, BIOPAC System, Inc. Santa Barbara, CA). Heart rate was determined from the intra-beat intervals.

2.2. Vascular reactivity measurements

After blood pressure and heart rate measurements, rats were sacrificed by exsanguination and the mesenteric arcade was carefully removed and placed in Krebs–Henseleit solution (KHS). With the use of a dissecting microscope, fourth-order mesenteric resistance arteries were carefully isolated, divided in segments of approximately 2 mm in length and mounted in a small vessel dual-chamber myograph containing KHS, gassed (95% O_2 and 5% CO_2) to maintain the pH at 7.4, at 37 °C, for measurement of isometric tension, according to the method described by Mulvany and Halpern (1977). Isometric tension recording was connected to an acquisition system (MacLab/41 ADInstruments Pty Ltd, Castle Hill, Australia).

After an equilibration period of 30 min, mesenteric resistance arteries were exposed to 120 mM potassium chloride (KCl) to check their functional integrity. Then, the presence of endothelium was confirmed by the effect of 10 μM acetylcholine on segments contracted with phenylephrine at a concentration that produces close to 75% of the contraction induced by 120 mM KCl. Afterwards, concentration–response curves to acetylcholine (0.1 nM–10 μM) were performed in segments previously contracted with phenylephrine at a concentration that produced approximately 75% of the maximal contraction induced by KCl. After a washout period of 45 min, a single concentration–response curve to phenylephrine (3 nM–30 μM) was constructed. The role of endothelium on phenylephrine responses was evaluated by removing this vascular component with a human hair; the effectiveness of endothelium removal was confirmed by the absence of relaxation induced by acetylcholine (10 μM) in precontracted arterial segments. In other set of experiments, the effects of the nonspecific NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) on the phenylephrine-induced responses were investigated, which was added 45 min before the second concentration–response curve to phenylephrine.

To evaluate the role of prostanoids or the EDHF on phenylephrine-induced contractions, three consecutive concentration–response curves to phenylephrine were performed at 1 h intervals. For this, L-NAME (100 μM) plus indomethacin (10 μM) and L-NAME plus indomethacin plus tetraethylammonium (TEA, 2 mM) were added 45 min before the second and the third concentration–response curve, respectively.

The functional activity of Na^+, K^+-ATPase in segments from control and ouabain-treated rats was measured using K^+-induced relaxation, as described by Webb and Bohr (1978) and modified by Rossoni et al. (1999). After a 30 min equilibration period in normal KHS, preparations were incubated during 30 min in a K^+-free KHS. Afterwards, the preparations were precontracted with phenylephrine (0.1 μM) and once a plateau was attained, the concentration of KCl was increased (1, 2, 4 and 6 mM) in steps each one with 2 min duration. After these procedures, preparations were incubated with 100 μM ouabain for 30 min and the K^+-induced relaxation curve was repeated.

2.3. Western blot analysis of eNOS and COX-2 protein expression

For analysis of eNOS and COX-2 expression, the second, third and fourth branches of superior mesenteric artery were dissected out, cleaned of connective tissue and frozen in liquid nitrogen and kept at −70 °C until the day of analysis. Proteins from homogenized mesenteric arteries (60 μg per lane) were separated by 7.5% SDS-PAGE and then transferred to polyvinyl difluoride membranes overnight. Next, the membrane was incubated for 2 h at room temperature with mouse monoclonal antibody for eNOS (1:2000, Transduction Laboratories, Lexington, UK) or COX-2 (1:1000; Cayman Chemical; Ann Arbor, MI, USA). After washing, membranes were incubated with

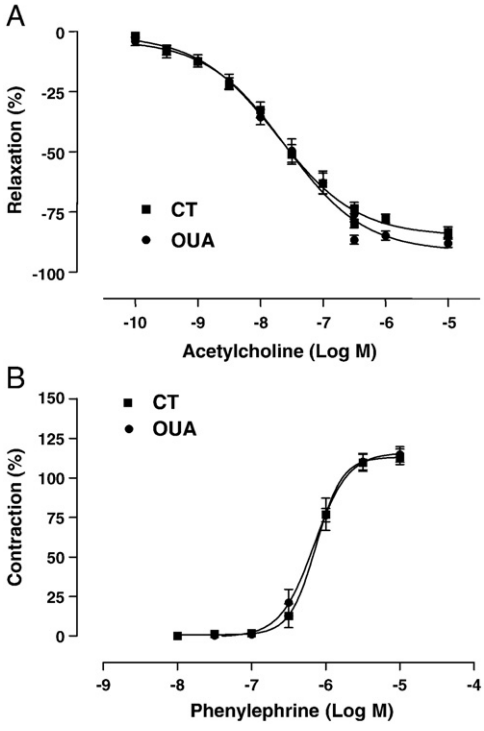

Fig. 1. Concentration–response curves to (A) acetylcholine and (B) phenylephrine in mesenteric resistance arteries from untreated (CT, $N=10, 8$) and ouabain-treated (OUA, $N=9, 7$) rats. Results are expressed as means ± S.E.M.