Vascular Pharmacology 58 (2013) 105–111

Contents lists available at SciVerse ScienceDirect

Vascular Pharmacology

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

Addition of adult serum improves endothelium-dependent relaxation of organ-cultured rat mesenteric artery via inhibiting mitochondrial reactive oxygen species

Tomoka Morita, Muneyoshi Okada, Yukio Hara, Hideyuki Yamawaki *

Laboratory of Veterinary Pharmacology, School of Veterinary Medicine, Kitasato University, Aomori 034-8628, Japan

ARTICLE INFO

Article history:
Received 22 May 2012
Received in revised form 31 July 2012
Accepted 26 August 2012

Keywords:
Serum
Antioxidant
Vascular endothelium
Organ culture
Mitochondria

ABSTRACT

Organ culture of blood vessels is a useful technique to investigate the long-term effects of drugs. Organ culture in a serum-free condition is so far the best way to maintain differentiated cell function. However some functional changes may occur from freshly isolated blood vessel (fresh) presumably due to lack of some key factors for vascular homeostasis in the medium. We investigated the long-term effects of addition of adult rat serum on acetylcholine-induced endothelium-dependent relaxation (EDR). Rat isolated mesenteric arteries were cultured for 3 days without (0% serum) or with 3% serum. In 0% serum, EDR was significantly impaired from fresh, whereas sodium nitroprusside-induced relaxation of smooth muscle didn't change. Addition of 3% serum significantly normalized the impaired EDR. Acute treatment with N-acetyl-L-cysteine or a mitochondrial inhibitor, rotenone normalized the impaired EDR in 0% serum. Mitochondrial superoxide production increased in the endothelium with 0% serum, which was normalized by 3% serum. Mitochondrial membrane potential increased in the endothelium with 0% serum, which was not normalized by 3% serum. In summary, the increased endothelial mitochondrial membrane potential in 0% serum may lead to mitochondrial reactive oxygen species (ROS) production and subsequent impairment of EDR. Addition of adult serum normalized the impaired EDR in part through inhibiting the increased mitochondrial ROS but not the membrane potential.

1. Introduction

Organ culture of blood vessels provides a useful method to investigate the long-term effects of drugs and/or physiologically active substances because tissue architecture and function are well preserved in the system (Ozaki and Karaki, 2002). So far, it was considered that organ culture in a serum-free condition is the best way to maintain differentiated cell function. In the recent study, we have established a serum-free organ-culture technique using rat isolated mesenteric artery (Morita et al., 2011, 2010). We have demonstrated that 3-day organ-cultured rat mesenteric arteries in a serum-free condition preserved an enough smooth muscle contractility to enable the analysis of long-term effects of drugs. By using this technique, we have found that long-term treatment of mesenteric artery with fetal bovine serum, a complex mixture of growth factors, attenuated agonist-induced smooth muscle contractility (Morita et al., 2010) as well as agonist-induced endothelium-dependent relaxation (Morita et al., 2011). In this way, organ-culture of blood vessels helps us to understand the roles of physiologically active substances in the vascular pathophysiological states (Kida et al., 2009, 2011; Mukohda et al., 2012; Murata et al., 2001a, 2001b, 2001c, 2004, 2005a, 2005b; Sakamoto et al., 2005; Yamawaki et al., 1999a, 1999b, 2000, 2012). However, compared with freshly isolated vessels, some functional changes such as decreased contractility, increased sensitivity to agonists and the changes in receptor expression level, may occur during a serum-free organ-culture (Cao et al., 2005, 2006; Luo et al., 2004, 2006; Morita et al., 2010; Tai et al., 2009; Uddman et al., 2003; Yamawaki et al., 2000).

Vascular endothelium usually contacts blood in in vivo situations. Various nutrients such as proteins, vitamins, and lipids in the blood are thought to be important to maintain vascular homeostasis. However, the medium used for a serum-free organ culture system may lack such nutritional factors. Therefore the aim of the present study was to investigate the long term effects of addition of adult rat serum which may involve such nutritional factors on endothelium-dependent relaxation (EDR) in serum-free organ-cultured rat isolated mesenteric artery. We have found that mitochondrial membrane potential increased in the endothelium during organ culture of rat mesenteric artery under serum-free condition for 3 days, which may lead to mitochondrial-derived reactive oxygen species (ROS) production and subsequent impairment of EDR. Addition of adult serum did not inhibit the increased mitochondrial membrane potential in 0% serum, but

* Corresponding author. Tel.: +81 176 23 4371; fax: +81 176 24 9456.
E-mail address: yamawaki@vmas.kitasato-u.ac.jp (H. Yamawaki).

1537-1891/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.vph.2012.08.006

normalized the impaired EDR at least in part through inhibiting mitochondrial ROS.

2. Materials and methods

2.1. Tissue preparation and organ culture procedure

Organ culture of rat isolated mesenteric artery was performed as described previously (Morita et al., 2010, 2011). In brief, male Wistar rats (6–12-week-old) were anesthetized with urethane (1.5 g/kg, i.p.) and euthanized by exsanguination. The main branch of the superior mesenteric artery was isolated under sterile conditions. After removal of fat and adventitia, the mesenteric artery was cut into rings (1-mm in diameter) for organ culture and measurement of isometric contraction. Blood was collected through the jugular vein and serum was obtained after centrifugation (4000 rpm, 5 min). The heat-inactivated (56 °C, 30 min) serum was sterilized using a 0.45 μm filter. Arterial rings were placed in 1 ml Dulbecco's Modified Eagle Medium (DMEM) without (0% serum) or with 3% rat serum (3% serum) supplemented with 1% penicillin–streptomycin. They were maintained at 37 °C in an atmosphere of 95% air and 5% CO_2 for 3 days. Animal care and treatment were conducted in conformity with institutional guidelines of the Kitasato University.

2.2. Measurement of isometric contraction

The arterial rings were placed in a normal physiological salt solution (PSS), which contained (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.5, $MgCl_2$ 1.0, $NaHCO_3$ 23.8, and glucose 5.5. Ethylenediaminetetraacetic acid (EDTA), 1 μM, was also added to remove the contaminating metal ions which catalyze the oxidation of organic chemicals. The high K^+ solution was prepared by replacing NaCl with equimolar KCl. These solutions were saturated with a 95% O_2–5% CO_2 mixture at 37 °C and pH 7.4. Smooth muscle contractility was recorded isometrically with a force-displacement transducer (Nihon Kohden, Tokyo, Japan) as described previously(Morita et al., 2010, 2011; Mukohda et al., 2010a, 2010b; Yamawaki et al., 2010). Each arterial ring was attached to a holder under a resting tension of 0.5 g. After equilibration for 30 min in a 3 ml organ bath, each ring was repeatedly exposed to a high K^+ (72 mM) solution until the responses became stable (60–90 min). Concentration–response curves were obtained by the cumulative application of relaxants during the

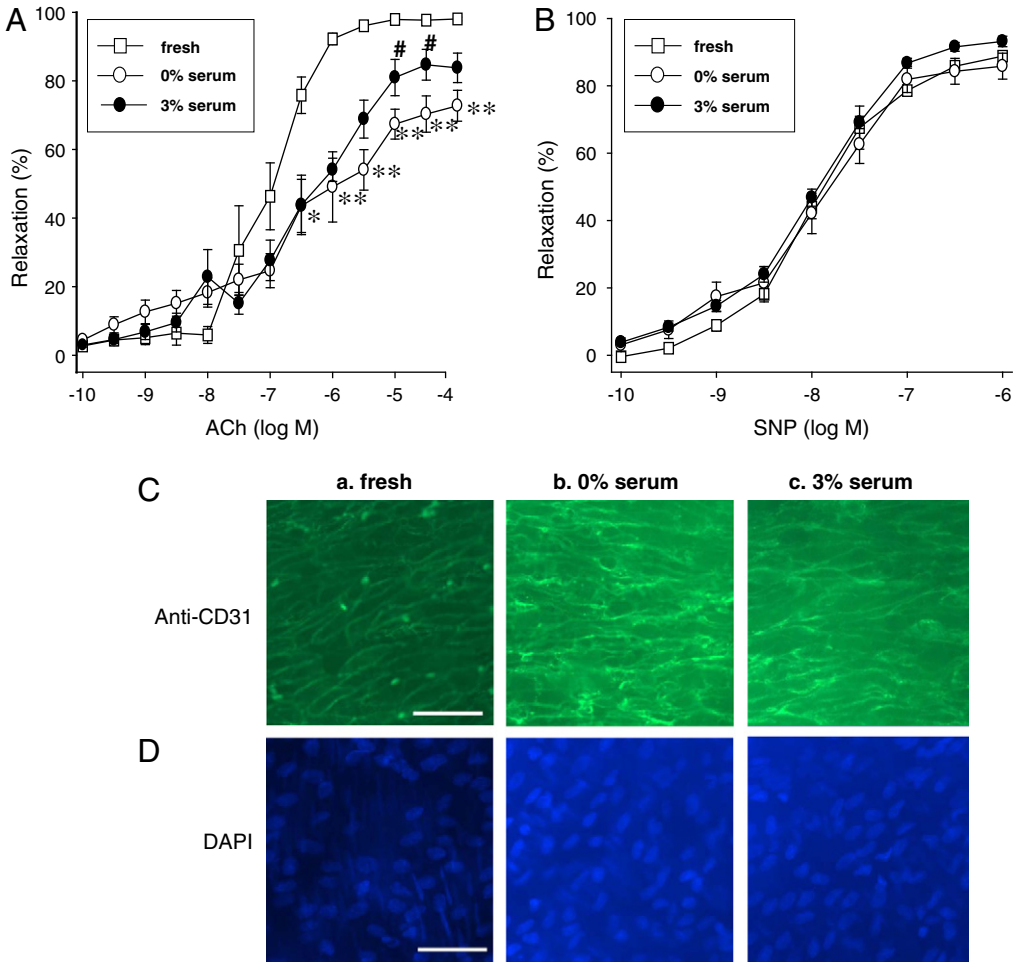

Fig. 1. Concentration–response relationships for relaxant effect of acetylcholine (ACh) (A: 0.1 nM–100 μM, n = 6 for fresh, n = 5 for 0% serum, n = 6 for 3% serum) and sodium nitroprusside (SNP) (B: 0.1–1000 nM, n = 6 for fresh, n = 6 for 0% serum, n = 6 for 3% serum) in endothelium-intact rat mesenteric arteries freshly isolated (fresh, open square) or cultured without (0% serum, open circle) or with 3% adult rat serum (3% serum, closed circle) for 3 days. The relaxants were cumulatively applied after the potassium-induced contraction had reached a steady state. Results are expressed as means ± S.E.M. Data was shown as a percent relaxation of the steady-state precontraction. *, **: $p<0.05$, 0.01, 0% serum vs. fresh. #: $p<0.05$, 3% serum vs. 0% serum. (C) Representative photomicrographs of en face immunohistochemistry against antibody to a specific endothelial cell marker, CD31 (anti-CD31, 1:500, overnight) in fresh, 0% serum, or 3% serum. (D) The arteries were nuclear stained with 4′,6-diamidino-2-phenylindole (DAPI, 1:1000, 5 min). Scale bar: 50 μm.