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European Journal of Pharmacology 539 (2006) 192–194
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Short communication

Upregulation of endothelin ET_B receptor-mediated vasoconstriction in rat coronary artery after organ culture

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Received 19 December 2005; received in revised form 20 April 2006; accepted 25 April 2006
Available online 3 May 2006

Abstract

The aim of this study was to examine if endothelin ET_B receptor-mediated contraction occurred in isolated segments of rat coronary arteries during organ culture. Presence of contractile endothelin ET_B receptors was studied by measuring the change in isometric tension in rings of left anterior descending coronary arteries isolated from hearts of rats as response to application of the selective endothelin ET_B receptor agonist, Sarafotoxin 6c and endothelin-1. In segments cultured 1 day in serum free Dulbecco's Modified Eagle's Medium, Sarafotoxin 6c induced a concentration dependent contraction with a pEC_{50} value of 10.4±0.21 and a maximal response of 3.9±0.25 mN/mm (n=15). The maximal contraction was significantly larger than the responses measured in fresh tissue, where the mean value of the maximal contractions was 0.22±0.03 mN/mm (n=17). The increased contraction to Sarafotoxin 6c after culturing could be eliminated with addition of the transcriptional blocker, actinomycin D, to the culture medium or be significantly attenuated by application of the translational inhibitor, cycloheximide. The vasoconstrictor effect of endothelin-1 or to depolarisation by high K^+-solution was not modified after 1 day in culture medium. The experiments indicate that organ culture of rat coronary arteries upregulate endothelin ET_B receptor-mediated contraction by inducing synthesis of new protein.

Keywords: Sarafotoxin 6c; Actinomycin D; Cycloheximide; Vasoconstriction

1. Introduction

Endothelin-1 belongs to a family of potent vasoactive hormones secreted from the endothelium (Yanagisawa et al., 1988). In blood vessels it is generally observed that constriction evoked by endothelin-1 is mediated by endothelin ET_A receptors on smooth muscle cells and dilatation via release of NO is mediated by endothelin ET_B receptors located on endothelial cells. This concept was demonstrated for the endothelin-1 effect on coronary flow in isolated rat heart, where endothelin-1 reduced coronary flow and application of an endothelin ET_B receptor antagonist enhanced the endothelin-1 evoked vasoconstriction in agreement with attenuation of vasodilatation by inhibition of endothelin ET_B receptors (Wang et al., 1994). The model has however been modified with the pharmacological demonstration that vasoconstriction could be induced by endothelin-1 in the presence of an endothelin ET_A antagonist and by application of the selective endothelin ET_B receptor agonist, Sarafotoxin 6c, indicating that vasoconstriction could also be mediated by endothelin ET_B receptors on smooth muscle cells (Balwierczak, 1993; Zhang et al., 1998). Furthermore endothelin ET_B receptors have been detected in both the endothelium and the smooth muscle cells in the rat coronary arteries by immunohistochemistry (Wendel-Wellner et al., 2002).

The degree of functional endothelin ET_B receptor expression in the smooth muscle cells may be the subject for modulation and is possibly related to pathophysiological conditions of the coronary vessels. Vasoconstriction mediated by endothelin ET_B receptors is for instance more prominent in older rats compared to young rats (Goodwin et al., 1999). Upregulation of endothelin ET_B receptors has been demonstrated in coronary arteries from patients with arteriosclerosis, and may be one of the consequences following ischemic heart disease and congestive heart failure

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doi:10.1016/j.ejphar.2006.04.024

(Cannan et al., 1996; Dagassan et al., 1996; Iwasa et al., 1999; Wackenfors et al., 2004).

The plasticity of endothelin ET_B receptor expression has previously been examined in peripheral and cerebral arteries from rat and omental arteries from human beings when cultured in serum free Dulbecco's Modified Eagle's Medium by demonstrating rapid upregulation of mRNA for endothelin ET_B receptors followed by induction of vasoconstrictive endothelin ET_B receptors in the smooth muscle cells (Adner et al., 1996; Moller et al., 1997; Adner et al., 1998; Hansen-Schwartz and Edvinsson, 2000). The aim of the present study was to show that organ culture of coronary arteries isolated from rat hearts induces endothelin ET_B receptor-mediated vasoconstriction and that the effect can be prevented by transcriptional and translational inhibitors at functional and mRNA levels.

2. Materials and methods

The investigation conforms within the national laws and guidelines for care and use of laboratory animals. 350–400 g male Sprague–Dawley rats (Taconic, Denmark) were anaesthetized with CO_2 and killed by decapitation. The chest of the animal was opened and the heart was rapidly excised and placed in ice-cold Krebs solution. The left anterior descending coronary artery was isolated and cut into segments of approximately 1 mm in length. The segments were mounted in myographs (DMT, Denmark) either immediately or placed in a 24 well culture dish containing serum free Dulbecco's Modified Eagle's Medium (Cambrex) supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml, GibcoBRL) and incubated overnight at 37 °C in an atmosphere of 5% CO_2 in air. Some segments were incubated with Actinomycin D (5 μg/ml, Sigma) or cycloheximide (10 μg/ml, Sigma).

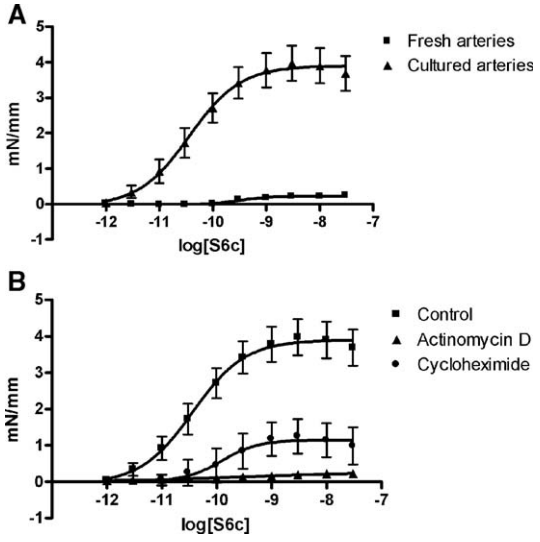

Fig. 1. Concentration–response curves for the Sarafotoxin 6c induced contraction in coronary arteries. Each point represents the mean±S.E.M. of all segments tested. A. Arteries incubated overnight in culture medium ($n=15$) and compared to responses in fresh arteries ($n=17$). $P<0.001$ (Bonferroni) for concentrations higher than 10^{-10} M Sarafotoxin 6c. B. The response of cultured coronary arteries incubated with actinomycin D ($n=6$, one-way ANOVA: $P<0.001$, Dunnett's post-test: $P<0.01$ at concentrations $\geq 10^{-10}$ M Sarafotoxin 6c) or cycloheximide ($n=4$, one-way ANOVA: $P<0.05$, Dunnett's post-test: $P<0.05$ at concentrations $\geq 10^{-10}$ M Sarafotoxin 6c) are reduced compared to the respective control ($n=17$).

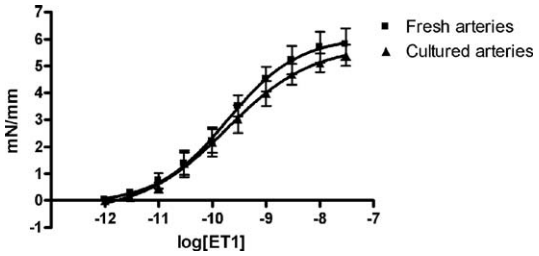

Fig. 2. Concentration–response curves for the ET-1 induced contraction in coronary arteries incubated overnight in DMEM ($pEC_{50}=9.73\pm0.62$, $E_{max}=5.76\pm0.62$ mN/mm, $n=7$) and compared to responses in fresh arteries ($pEC_{50}=9.72\pm0.18$, $E_{max}=6.08\pm0.50$ mN/mm, $n=7$). Each point represents the mean±S.E.M. of all segments tested.

The organ baths containing Krebs solution are temperature controlled (35–37 °C) and constantly bubbled with 95% O_2/5% CO_2. Measurement of tension is recorded by Chart-software (AD-Instrument, Denmark) using the PowerLab/SP8 from AD instruments as analog-digital converter. For a segment length of 1 mm an initial wall force of 2 mN was applied. The vessels were subsequently allowed to stabilize for 1 h before the isometric tension was measured as response to application of isotonic high K^+–Krebs solution to test the viability of segments. Concentration–response curves were performed by cumulative addition of the drugs.

The composition of the Na–Krebs solution, in mM, was as follows: NaCl: 119, KCl: 4.6, $NaHCO_3$: 15, $NaHPO_4$: 15, $MgCl_2$: 1.2, $CaCl_2$: 1.5 glucose: 5.5. In the high K–Krebs solution 60 mM of the NaCl was replaced with isotonic amount of KCl. Endothelin-1 and Sarafotoxin 6c (AnaSpec, USA) were dissolved in distilled water containing 0.1% (w/v) bovine serum albumin. Krebs-solution containing 0.1% bovine serum albumin was used for dilution of endothelin-1.

All values are given as mean±S.E.M. EC_{50} (molar concentration of the agonist that produced half maximal response) and E_{max} (maximal response) were calculated by non-linear regression with GraphPad Prism 4 (GraphPad, San Diego, CA, U.S.A). Differences of the mean values were tested for statistical significance using *t*-test followed by Bonferroni's post-test for selected pairs and one-way ANOVA followed by Dunnett's post-test for multiple comparisons.

3. Results

In control experiments on fresh coronary arteries, Sarafotoxin 6c induced a slight contraction in 9 out of 17 segments. The mean value of the maximum responses is 0.22±0.03 mN/mm ($n=17$). Organ culture of the arteries induced Sarafotoxin 6c sensitive vasoconstriction in all segments tested. The dose–response curve for Sarafotoxin 6c gave a pEC_{50} of 10.4±0.21 and a maximal response E_{max} of 3.9±0.25 mN/mm ($n=15$) (Fig. 1A). Organ culture did not affect the ability of the smooth muscles to contract as response to high K^+-solution. The mean value of the K^+-response in fresh segments was 2.57±0.26 mN/mm ($n=24$) and in

cultured segments 2.64±0.30 mN/mm (n=22). The Sarafotoxin 6c response was eliminated when the segments were incubated with the transcriptional inhibitor actinomycin D (5 μg/ml), giving a maximal response of 0.30±0.17 mN/mm (Fig. 1B). It was significantly reduced when the culture medium is supplied with 10 μg/ml of the translational inhibitor cycloheximide giving a $p\mathrm{EC}_{50}$=9.9±0.4 and a maximal response of 1.15±0.21 mN/mm (Fig. 1B).

In order to examine if induction of endothelin ET_B receptors in smooth muscle cells modifies the vasoconstrictor effect to the endogenous transmitter, dose–response curves for endothelin-1 were performed. No significant difference in the concentration–response curve between the fresh ($p\mathrm{EC}_{50}$=9.72±0.18, E_{max}=6.08±0.50 mN/mm) and the cultured arteries ($p\mathrm{EC}_{50}$=9.73±0.62, E_{max}=5.76±0.62 mN/mm) was observed (Fig. 2). The dose–response curves fitted to the experimental results gave a Hill coefficient less than 1 indicating deviation from a one-site model. However attempts to fit the curves to a two-site model did not give meaningful results.

4. Discussion

This study has shown plasticity of a receptor in the coronary arteries. The endothelin ET_B receptor agonist, Sarafotoxin 6c, only induced minor contraction in freshly dissected coronary arteries but having been cultured in serum free Dulbecco's Modified Eagle's Medium for more than 20 h significant contractile response to Sarafotoxin 6c was demonstrated. The ability of the smooth muscles to contract as response to potassium depolarisation had not changed as a result of culturing. The event appears to be coupled to newly transcribed mRNA since the transcriptional blocker actinomycin D could prevent the effect. Reduced response to Sarafotoxin 6c could also be demonstrated in vessels cultured with the translational inhibitor cycloheximide. The phenomenon is in accordance with previous studies on rat mesenteric, rat cerebral and human omental arteries, where upregulation of mRNA for endothelin ET_B receptors was also demonstrated, an effect that occurred prior to registration of the functional effect (Adner et al., 1996; Adner et al., 1998; Moller et al., 1998; Hansen-Schwartz and Edvinsson, 2000; Moller et al., 2002; Henriksson et al., 2003). The coronary artery from rat is a new vessel where endothelin ET_B receptor-mediated vasoconstriction is demonstrated.

Since induction of endothelin ET_B receptor-mediated contraction may be a response of the arteries under experimental conditions and result in modified vascular tone, it was examined if vasoconstriction induced by the endogenous transmitter endothelin-1 was changed. It has previously been shown that arteries that have been cultured for 2 days may show increased sensitivity of receptor activated contractions as demonstrated for endothelin in cerebral arteries (Hansen-Schwartz and Edvinsson, 2000). In mesenteric arteries one study did not measure any change in potency for endothelin but the maximal response was increased after 1 day and decreased after 2 days in culture medium (Moller et al., 1997). In another study a significantly higher EC_{50} value for Sarafotoxin 6c was measured after 48 h culturing compared to 24 h (Moller et al., 2002). In this study we could not detect any difference in potency or maximal response to endothelin between the one-day cultured arteries and the fresh arteries. The response of coronary arteries to endothelin after 2 days in culture medium was not examined.

The experiments show that culturing of isolated coronary arteries from rats induces endothelin ET_B receptor-mediated vasoconstriction. It requires, however, further experiments to demonstrate if this is due to an upregulation of the ET_B receptors in the smooth muscle cells or to induction of some other factors that is needed to activate already existing receptors.

References

Adner, M., Cantera, L., Ehlert, F., Nilsson, L., Edvinsson, L., 1996. Plasticity of contractile endothelin-B receptors in human arteries after organ culture. Br. J. Pharmacol. 119, 1159–1166.

Adner, M., Geary, G.G., Edvinsson, L., 1998. Appearance of contractile endothelin-B receptors in rat mesenteric arterial segments following organ culture. Acta Physiol. Scand. 163, 121–129.

Balwierczak, J.L., 1993. Two subtypes of the endothelin receptor (ETA and ETB) mediate vasoconstriction in the perfused rat heart. J. Cardiovasc. Pharmacol. 22 (Suppl 8), S248–S251.

Cannan, C.R., Burnett Jr., J.C., Lerman, A., 1996. Enhanced coronary vasoconstriction to endothelin-B-receptor activation in experimental congestive heart failure. Circulation 93, 646–651.

Dagassan, P.H., Breu, V., Clozel, M., Kunzli, A., Vogt, P., Turina, M., Kiowski, W., Clozel, J.P., 1996. Up-regulation of endothelin-B receptors in atherosclerotic human coronary arteries. J. Cardiovasc. Pharmacol. 27, 147–153.

Goodwin, A.T., Amrani, M., Marchbank, A.J., Gray, C.C., Jayakumar, J., Yacoub, M.H., 1999. Coronary vasoconstriction to endothelin-1 increases with age before and after ischaemia and reperfusion. Cardiovasc. Res. 41, 554–562.

Hansen-Schwartz, J., Edvinsson, L., 2000. Increased sensitivity to ET-1 in rat cerebral arteries following organ culture. NeuroReport 11, 649–652.

Henriksson, M., Stenman, E., Edvinsson, L., 2003. Intracellular pathways involved in upregulation of vascular endothelin type B receptors in cerebral arteries of the rat. Stroke 34, 1479–1483.

Iwasa, S., Fan, J., Shimokama, T., Nagata, M., Watanabe, T., 1999. Increased immunoreactivity of endothelin-1 and endothelin B receptor in human atherosclerotic lesions. A possible role in atherogenesis. Atherosclerosis 146, 93–100.

Moller, S., Edvinsson, L., Adner, M., 1997. Transcriptional regulated plasticity of vascular contractile endothelin ET(B)receptors after organ culture. Eur. J. Pharmacol. 329, 69–77.

Moller, S., Adner, M., Edvinsson, L., 1998. Increased levels of endothelin ETB receptor mRNA in human omental arteries after organ culture: quantification by competitive reverse transcription-polymerase chain reaction. Clin. Exp. Pharmacol. Physiol. 25, 788–794.

Moller, S., Uddman, E., Welsh, N., Edvinsson, L., Adner, M., 2002. Analysis of the time course for organ culture-induced endothelin ET B receptor upregulation in rat mesenteric arteries. Eur. J. Pharmacol. 454, 209–215.

Wackenfors, A., Emilson, M., Ingemansson, R., Hortobagyi, T., Szok, D., Tajti, J., Vecsei, L., Edvinsson, L., Malmsjo, M., 2004. Ischemic heart disease induces upregulation of endothelin receptor mRNA in human coronary arteries. Eur. J. Pharmacol. 484, 103–109.

Wang, Q.D., Li, X.S., Pernow, J., 1994. Characterization of endothelin-1-induced vascular effects in the rat heart by using endothelin receptor antagonists. Eur. J. Pharmacol. 271, 25–30.

Wendel-Wellner, M., Noll, T., Konig, P., Schmeck, J., Koch, T., Kummer, W., 2002. Cellular localization of the endothelin receptor subtypes ET(A) and ET (B) in the rat heart and their differential expression in coronary arteries, veins, and capillaries. Histochem. Cell Biol. 118, 361–369.

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K., Masaki, T., 1988. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332, 411–415.

Zhang, Y., Oliver, J.R., Horowitz, J.D., 1998. Endothelin B receptor-mediated vasoconstriction induced by endothelin A receptor antagonist. Cardiovasc. Res. 39, 665–673.