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Decreased vasorelaxation induced by iloprost during acute inflammation in human internal mammary artery

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ABSTRACT

Cyclooxygenase-2 (COX-2) induction in human internal mammary arteries (IMA) under inflammatory conditions has been associated with attenuated norepinephrine (NE)-induced vasoconstriction. This effect was associated with increased prostaglandin (PG) E_2 and prostacyclin (PGI_2) releases. The present study was designed to assess the role of these PG and their receptors (EP and IP, respectively) on the vascular reactivity during acute inflammation. Isolated IMA were cultured in the absence (Control conditions) or presence (Inflammatory conditions) of both interleukin-1 beta (IL-1β) and lipopolysaccharide (LPS). The vasorelaxation and the increased content of cyclic adenosine monophosphate (cAMP) induced by iloprost, a PGI_2 analogue, were significantly reduced under inflammatory conditions and restored in preparations cultured with the IP antagonist (CAY10441). Decreased cAMP levels under inflammatory conditions are due to at least increased phosphodiesterase (PDE) 4B expression. On the other hand, PGE_2, thromboxane analogues and EP agonists-induced vasoconstrictions were not affected under inflammatory conditions. No vasorelaxation was observed with PGD_2, PGE_2 or the EP2/4 agonists in pre-contracted IMA. Finally, using RT-qPCR and immunohistochemistry, the COX-2, IP receptor and PGI_2 synthase (PGIS) were detected. A significant increase of COX-2 and moderate increase of IP mRNA expression was observed under inflammatory conditions, whereas PGIS mRNA level was not affected. This study demonstrates that PGI_2/IP receptor signalling and PGI_2-induced relaxation are impaired in human IMA during acute inflammation, whereas the responses induced by other prostanoids are not affected. These results could explain some of the mechanisms of vascular dysfunction reported in inflammatory conditions.

1. Introduction

Prostanoids [prostaglandins (PG) and thromboxane (Tx)] regulate a broad range of (patho)physiological processes such as inflammation and vascular reactivity (Foudi et al., 2012; Gomez et al., 2013; Norel, 2007). Prostanoids derive from arachidonic acid via the cyclooxygenase (COX) pathway. Two isoforms of this enzyme have been described: COX-1, the constitutive isoform present in physiological conditions and COX-2, an inducible isoform, which is expressed during Inflammatory conditions and under pathological situations such as atherosclerosis and aneurysm (Cipollone et al., 2005; Gomez et al., 2013; Wang et al., 2008). Both COX isoforms initiate a metabolic cascade leading to the formation of PGH_2 from arachidonic acid. PGH_2 is converted by isomerases such as PGI synthase (PGIS) to prostacyclin (PGI_2).

Many prostanoids play a major role in the control of vascular tone by the activation of their specific receptors. For instance, PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and TxA_2 activate preferentially DP, EP1-4, FP, IP and TP receptors, respectively, as agreed by the NC-IUPHAR Subcommittee on Prostanoid Receptors (Alexander et al., 2015). The activation of IP causes vasorelaxation mediated by an increase of cyclic adenosine monophosphate (cAMP) cellular content via G protein and adenylate cyclase-dependent signalling (Norel, 2007; Norel et al., 2004). Among 11 known families of phosphodiesterases (PDE), it has been shown that PDE3 and PDE4, responsible for degradation of cAMP, are expressed

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in vascular preparations (Houslay et al., 2007; Santos-Silva et al., 2008).

Endothelial and smooth muscle cells derived from human vessels can preferentially secrete PGE_2 and PGI_2. The release of these PGs was increased in cells exposed to proinflammatory stimuli such as interleukin (IL)-1β and lipopolysaccharide (LPS) (Camacho et al., 2007; El-Haroun et al., 2004; Jang et al., 2015). In addition, prostanoid receptors have been induced in several inflammatory conditions such as atherosclerosis and arterial aneurysm (Bayston et al., 2003; Cipollone et al., 2005; Gomez et al., 2013) and in cells after inflammatory stimuli (Takayama et al., 2006).

The use of COX-2 inhibitors (COXIBs) has been associated with an increased risk of acute complications of cardiovascular disease in patient with risk factors/documented atheroma. The decrease of prostanoids secretion due to COX-2 inhibition also leads to the alteration of the vascular tone. All these results underline the major role played by PG and their receptors in the regulation of vascular biology under physiological/pathophysiological conditions.

In a previous study we demonstrated that COX-2 induced under Inflammatory conditions decreased norepinephrine (NE) reactivity of isolated human internal mammary arteries (IMA) (Foudi et al., 2009a). This effect was associated with a two fold increase in PGE_2 and PGI_2 synthesis but the consequences of this phenomenon on the vascular tone under these conditions are not well documented. Furthermore, the expression/function of the receptors activated by these PGs in IMA could be also modified under Inflammatory conditions. In this context, the aims of this study were: (i) to investigate the effects on vascular tone of each PG and selective agonists/antagonists of the respective receptors; (ii) to test the hypothesis that there are specific changes in the cognate receptors expression.

2. Materials and methods

2.1. Human vascular preparations

Human IMA were obtained from patients undergoing coronary artery bypass surgery (n=45; 38 males and 7 females, aged 65 ± 4 years old) at the Cardiovascular department, Bichat hospital (Paris, France). The investigation conforms to the principles outlined in the Declaration of Helsinki as these tissues were anonymized (rendered non-identifiable). All research programs involving the use of human tissue were approved and supported by the INSERM Ethics Committee and these tissues are considered as surgical waste in accordance with French ethical laws (L.1211–3 –L.1211–9).

2.2. Organ culture

The IMA were dissected free from connective tissue and placed immediately into 12-well plates containing RPMI (Roswell Park Memorial Institute) medium (Gibco, Waltham, MA USA; pH 7.4) supplemented with antibiotics (penicillin, 1000 IU/ml; streptomycin, 100 µg/ml) and an antimycotic (amphotericin, 0.25 µg/ml). All tissue incubations were performed at 37 °C in a humidified atmosphere of 5% CO_2 in air using a culture incubator. The volume of the culture medium was adjusted to 1 ml for 70 mg of tissue. IMA preparations were cultured in the absence (Control condition) or presence (Inflammatory condition) of both IL-1β (100 ng/ml) and LPS (Escherichia coli, 100 µg/ml) either for 24 h or 18 h (duration depended on the protocols; however, similar induction of inflammation and COX-2 in IMA was described in one of our previous publications after 6 h or 24 h incubation (Foudi et al., 2009a). This model of inflammation has been widely used in vascular tissues or cells at different incubation times (Bishop-Bailey et al., 1997, 1998; Camacho et al., 2011).

The selective IP antagonist (CAY10441, 1 µmol/l) was used in some protocols during this incubation period. Subsequent to this exposure, segments of the vascular preparations were placed in organ bath to measure vascular reactivity, whereas other segments were used for PCR analysis and immunohistochemistry.

2.3. Organ bath and isometric measurements

Human IMA preparations were cut as rings of 3 mm of width and set up in 10 ml organ baths containing Tyrode's solution (concentration mmol/l): NaCl 139.2, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.49, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.5, gassed with 5% CO_2 and 95% O_2 at 37 °C and pH 7.4. Each ring was initially stretched to optimal load ~1.5 g. Changes in force were recorded by isometric force displacement transducer (Narco F-60, USA) and Physiographs (Linseis, Germany). Rings were then equilibrated for 90 min with bath fluid changes taking place every 10 min. An initial contraction was performed with the NE (10 µmol/l). When the maximal effect was obtained, the preparations were washed with fresh Tyrode's solution until they returned to the resting tone. In order to avoid any physiological effect induced by endogenous PG and nitric oxide (NO), rings were incubated (30 min) with Tyrode's solution containing the COX inhibitor indomethacin (Indo, 1.7 µmol/l) and the NO-synthase inhibitor, NG-nitro-L-arginine (L-NOARG, 0.1 mmol/l). Some of these rings were exposed to selective antagonists (BAY u3405 10 µmol/l, CAY10441 1 µmol/l or L-826266 0.3 µmol/l) during this 30 min incubation period. Subsequently and without any bath fluid wash, concentration-responses were performed with prostanoids agonists (1 nmol/l -10 µmol/l). At the end of some protocols and in absence of response, a contraction with NE (10 µmol/l) or a relaxation with papaverine (0.1 mmol/l) were induced in order to verify the viability of tissues. Vascular rings were pre-contracted with NE (10 µmol/l) or the TP agonist U46619 (0.1 µmol/l) before concentration-responses for vasorelaxation protocols.

2.4. Measurement of cAMP content

Some vascular rings were co-incubated (18 h in Control conditions in a culture incubator, 70 mg tissue/ml) with or without (IL-1β+LPS) and with or without CAY10441 (1 µmol/l). After this incubation period, the preparations were rinsed 3 times before a 30 min stimulation in incubator with or without iloprost (1 µmol/l). At the end, each ring was snap frozen in nitrogen and then homogenized. The homogenate (100 mg) was resuspended in 1 ml of HCl (0.1 mol/l), centrifuged (180*g*, 30 min, 4 °C), the supernatant was harvested and frozen. Quantification of cAMP in the supernatant was performed using an EIA kit (Cayman n°581001).

2.5. Real time PCR

Human IMA preparations cultured under Control and Inflammatory conditions, as described in the Section 2.2, were disrupted using a Polytron apparatus (Kinematika, Luzern, Switzerland) and RNA was isolated using NucleoSpin RNA II Kit (Macherey-Nagel, Hoerdt, France). One microgram of total RNA was then reverse transcribed using Moloney murine leukemia virus reverse transcriptase and random primers (Invitrogen Life Technologies, Cergy Pontoise, France), and the cDNAs were subjected to real-time PCR using a LightCycler 480 Roche QPCR (Roche Diagnostics, Meylan, France). Real-time PCR were conducted in triplicate, using the following set of primers: COX-2 sense 5′- cttcacgcatcagtttttcaag -3′, and antisense 5′- tcaccgtaaatatgatttaagtccac -3′; IP sense 5′- cacgaggagcaaagcaagtg -3′, and antisense 5′- aggtctgggctctccagtctt -3′; PGIS sense 5′- aaaaggccaggatgaaactgact -3′, and antisense 5′- tctgtagcatcgcccaacag -3′; -3′. PDE 3A Sense 5′- aaagacaagcttgctattccaaa -3′ and antisense 5′- gtggaagaaactcgtctcaaca -3′; PDE 3B Sense 5′- aaaggggatagaaaacttaacaagg -3′ and antisense 5′-caggtagcaatcctgaagttcc -3′; PDE 4B Sense 5′- cctttctacaccagcattagacg -3′ and antisense 5′-ggatggcagctgcaaaaa -3′; PDE 4D Sense 5′- gctgcagatgttgtccagtc – 3′ and antisense 5′- ggcaaaaattgctgcaaga – 3′; S14 sense 5′- ccgatttctgattctaacaggac -3′,