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An in vitro reconstitution system to address the mechanism of the vascular expression of the bradykinin B_1 receptor in response to angiotensin converting enzyme inhibition

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ABSTRACT

The expression of the bradykinin (BK) B_1 receptor (B_1R), lacking in normal vascular tissues, is induced following innate immune system activation and chronic blockade of angiotensin converting enzyme (ACE). To identify cytokine-dependent or -independent mechanisms for the latter phenomenon, the ACE inhibitor enalaprilat and several peptides potentiated in vivo by ACE blockade were applied either directly to human umbilical artery smooth muscle cells (hUA-SMCs) or to differentiated monoblastoid U937 cells to produce a conditioned medium (CM) that was later transferred to hUA-SMCs. A phagocyte stimulant, lipopolysaccharide, did not upregulate B_1R, measured using [^{3}H]Lys-des-Arg^9-BK binding, or translocate NF-κB to the nuclei if applied directly to the hUA-SMCs. However, the CM of lipopolysaccharide-stimulated U937 cells was active in these respects (effects inhibited by etanercept and correlated to TNF-α presence in the CM). A peptidase-resistant B_1R agonist had no significant direct or indirect acute effect (4 h) on B_1R expression, but repeated hUA-SMC stimulations over 40 h were stimulatory in the absence of NF-κB activation. Other peptides regulated by ACE or enalaprilat did not directly or indirectly stimulate B_1R expression. The reconstitution system supports the rapid cytokine-dependent vascular induction of B_1Rs and a slow "autoregulatory" one potentially relevant for the ACE blockade effect.

1. Introduction

Bradykinin (BK)-related peptides, the kinins, stimulate two related G protein coupled receptors, the widely distributed and preformed B_2 receptor (B_2R) and the B_1 receptor (B_1R), responsive to kinin des-Arg^9 metabolites generated by arginine carboxypeptidases (Moreau et al., 2005b). Vasodilation, increased vascular permeability and smooth muscle cell (SMC) stimulation are mediated by both B_1R and B_2R subtypes, provided that they are expressed. Indeed, the B_1R is generally absent from vascular tissues of healthy animals, but the most documented systems where its expression is induced are related to immunopathology, e.g. following administration of bacterial lipopolysaccharide (LPS) in vivo. B_1R expression then proceeds under the influence of various cytokines, mitogen-activated protein kinases and NF-κB signaling (Larrivée et al., 1998; Marceau et al., 1998; Sabourin et al., 2002; Moreau et al., 2005b). In addition, limited but intriguing evidence has associated B_1R expression to chronic inhibition of angiotensin I converting enzyme (ACE). There is evidence in 3 animal species that B_1R expression is triggered by chronic (≥7 days), but not acute (≤48 h) ACE blockade in healthy subjects without obvious deleterious effect (Marin-Castano et al., 2002; Moreau et al., 2005a; Bawolak et al., 2008). Notably, in the porcine model, there was no evidence of systemic inflammation (fever, leukocyte count alteration, acute phase protein release) parallel to B_1R expression (Moreau et al., 2005a). The phenomenon may be relevant for both the therapeutic and side effects of ACE inhibitors.

ACE is an ectopeptidase expressed by vascular endothelial and other cells that regulates several humoral systems in vivo and that is an important drug target. ACE inhibitors are nowadays exploited in the therapy of hypertension, congestive heart failure, diabetic nephropathy and other ailments (Hanif et al., 2010). In dissecting which effect of ACE inhibitors may be relevant for B_1R expression, one must consider their mode of action. Their primary effect may derive from the inhibition of the formation of the vasoconstrictor octapeptide angiotensin (Ang) II from Ang I. As ACE is a prominent BK-destroying enzyme in vascular endothelial cells and the kidney

Abbreviations: ACE, angiotensin converting enzyme; Ang, angiotensin; B_1R, B_1 receptor; B_2R, B_2 receptor; B-9972, D-Arg-[trans-4-hydroxyprolyl^3, α-(2-indanyl)glycyl^5, (3as, 7as)-octahydroindol-2-yl-carbonyl^7, α-(2-indanyl)glycyl^8]-bradykinin; BK, bradykinin; CGRP, calcitonin gene related peptide; CM, conditioned medium; hUA-SMC, human umbilical artery smooth muscle cell; HUVEC, human umbilical vein endothelial cell; IFN, interferon; IL, interleukin; IRA, interleukin-1 receptor antagonist; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; PMA, phorbol 12-mysistate 13-acetate; Sar, sarcosine; SMC, smooth muscle cell; TNF-α, tumor necrosis factor-α.

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(Moreau et al., 2005b), there is evidence for a vasodilator component of the effect of ACE inhibitors derived from the potentiation of BK action on its widely expressed B_2Rs in acute clinical studies (Gainer et al., 1998; Pretorius et al., 2003; Squire et al., 2000). The endogenous B_1R agonists, des-Arg^9-kinins, also may increase during ACE blockade because they are formed by competing peptidase pathways and also because ACE inactivates them (Décarie et al., 1996). Further, ACE degrades Ac-Ser-Asp-Lys-Pro, an acetylated tetrapeptide derived from thymosin β4 (Fleming, 2006). ACE blockade also promotes the formation of the Ang_{1-7} fragment by diverting the metabolism of Ang I from the formation of Ang II (Santos et al., 2010). Both Ang_{1-7} and Ac-Ser-Asp-Lys-Pro are endowed with anti-inflammatory and anti-fibrotic activities, the former peptide via the G protein coupled receptor mas, and the latter by interacting with unknown molecular/cellular partner (Sharma et al., 2008). Peptides released by afferent nerve terminals, substance P and calcitonin gene related peptide (CGRP), may increase during ACE blockade, either because substance P is an ACE substrate (Fleming, 2006), or because these nerves are activated by endogenous BK (Fox et al., 1996). Finally, the ACE inhibitors, small molecular weight conventional drugs, may elicit an unconventional signaling response mediated by ACE itself. This protein, possessing a small intracellular tail that may be modified by phosphorylation events, may recruit certain signaling molecules (Fleming, 2006).

The general hypothesis tested in this study was that one of the humoral factor influenced by ACE blockade, including endogenous kinins, or an ACE inhibitor itself could induce the expression of B_1Rs in vascular cells via a cytokine-dependent or -independent mechanism. We have exploited the primary cultures of human umbilical artery smooth muscle cells (hUA-SMC), a well characterized model for B_1R regulation by multiple signaling pathways (Morissette et al., 2006; Moreau et al., 2007; Koumbadinga et al., 2010b), to address the mechanism of ACE inhibitor-induced induction of B_1Rs. Tissue macrophages may be more responsive to innate immune system stimuli than vascular cells and may somehow convert ACE blockade into inflammatory signaling. An ACE inhibitor and several peptides known to be potentiated in vivo by ACE blockade (Ang I, Ang_{1-7}, kinins, Ac-Ser-Asp-Lys-Pro, substance P, CGRP), and Ang II

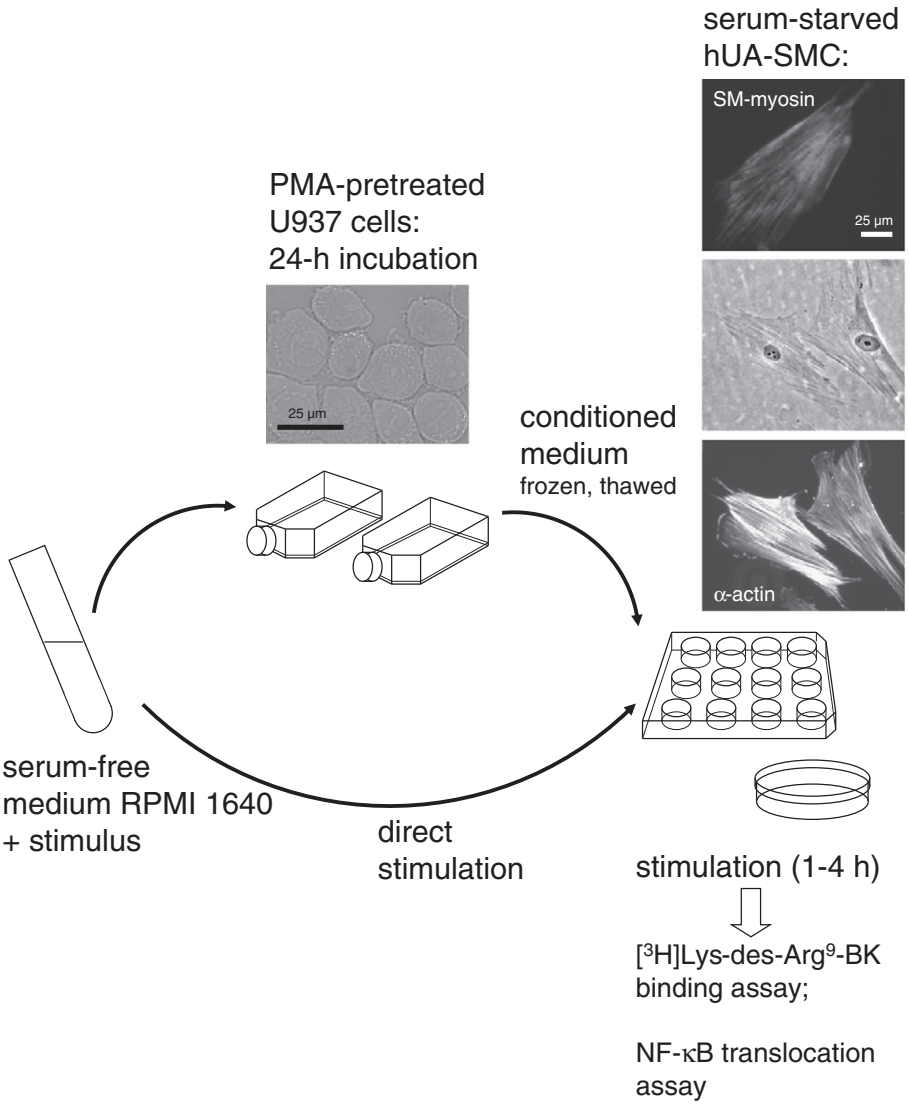

Fig. 1. Schematic representation of the protocol applied for the generation of conditioned RPMI-1640 medium from PMA-pretreated U937 cells or of medium containing the test substances for the direct stimulation, both of which were used to stimulate the expression of inflammatory genes in human vascular cells. Insets: appearance of adherent U937 cells (phase contrast) and of hUA-SMCs (phase contrast and immunofluorescence for α-actin, positive for virtually all cells, and smooth muscle myosin heavy chain, positive in less than 10% of cells). 25-μm scale bar indicated for each cell type.