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Vasopeptidase-activated latent ligands of the histamine receptor-1

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ABSTRACT

Whether peptidases present in vascular cells can activate prodrugs active on vascular cells has been tested with 2 potential latent ligands of the histamine H_1 receptor (H_1R). First, a peptide consisting of the antihistamine cetirizine (CTZ) condensed at the N-terminus of ε-aminocaproyl-bradykinin (εACA-BK) was evaluated for an antihistamine activity that could be revealed by degradation of the peptide part of the molecule. CTZ-εACA-BK had a submicromolar affinity for the BK B_2 receptor (B_2R; IC_{50} of 590 nM, [^{3}H]BK binding competition), but a non-negligible affinity for the human H_1 receptor (H_1R; IC_{50} of 11 μM for [^{3}H]pyrilamine binding). In the human isolated umbilical vein, a system where both endogenous B_2R and H_1R mediate strong contractions, CTZ-εACA-BK exerted mild antagonist effects on histamine-induced contraction that were not modified by omapatrilat or by a B_2R antagonist that prevents endocytosis of the BK conjugate. Cells expressing recombinant ACE or B_2R incubated with CTZ-εACA-BK did not release a competitor of [^{3}H]pyrilamine binding to H_1Rs. Thus, there is no evidence that CTZ-εACA-BK can release free cetirizine in biological environments. The second prodrug was a blocked agonist, L-alanyl-histamine, potentially activated by aminopeptidase N (APN). This compound did not compete for [^{3}H]pyrilamine binding to H_1Rs. The human umbilical vein contractility assay responded to L-alanyl-histamine (EC_{50} 54.7 μM), but the APN inhibitor amastatin massively (17-fold) reduced its apparent potency. Amastatin did not influence the potency of histamine as a contractile agent. One of the 2 tested latent H_1R ligands, L-alanyl-histamine, supported the feasibility of pro-drug activation by vascular ectopeptidases.

1. Introduction

Peptidases expressed in vascular tissue are important modulators of the pharmacology of vasoactive peptides. Angiotensin converting enzyme (ACE), expressed in endothelial cells, hydrolyses both angiotensin I and bradykinin (BK). In the first case, this is a physiological activation, as the product angiotensin II is the optimal agonist of the AT_1 receptors, while the second reaction leads to BK inactivation [1]. However, we reported lately that the peptide Met-Lys-bradykinin-Ser-Ser is paradoxically activated by its reaction with ACE, that frees the BK C-terminal sequence needed to activate the cognate B_2 receptor (B_2R) [2]. The modulatory role of vascular aminopeptidase N (APN) has also been illustrated, as the blockade of this ectopeptidase, expressed in vascular smooth muscle, potentiates such peptides as Lys-des-Arg^9-BK (the optimal agonist of human and rabbit BK B_1 receptor), some peptide antagonists of B_1 receptors and angiotensin III [3,4].

BK is a fragile peptide inactivated rapidly both in the extracellular compartment and in endosomes. The nonapeptide BK, via its preformed, phosphorylable and G protein coupled B_2R, is an excellent example of an agonist submitted to endocytosis and degradation [5]. Endosomal BK degradation obligatorily precedes the dissociation of β-arrestins from the B_2R and receptor recycling to the cell surface, as shown by several inactivation-resistant B_2R agonists that promote the persistence of this intracellular complex for at least 12 h and prolonged signaling [6,7]. Fluorophore conjugated analogs (carboxyfluorescein- or AlexaFluor-350-ε-aminocaproyl-BK) model the intracellular inactivation of the kinin, notably because the inhibitor of the proton pump V-ATPase, bafilomycin A1, prevents the time-dependent disappearance of the fluorescent peptides in endosomes of B_2R-expressing cells [8,9]. Free carboxyfluorescein is also released into the cytosol as a function of time in these cells, suggesting a particular strategy to release a drug cargo from BK conjugates.

A possible manner to generate diversity in drug candidates is to design ligands for more than one pharmacological target; for instance omapatrilat has been designed to block both BK-destroying peptidases ACE and neutral endopeptidase (NEP) with nanololar affinities [10]. A further step could concern ligands that successively bind to one target, and then to another upon metabolic alteration. Among several carboxylic acids that have been condensed with ε-aminocaproyl-BK, we recently reported the antihistamine drug cetirizine (Fig. 1) [9]. As other N-terminally extended analogs of BK, cetirizine-ε-aminocaproyl-bradykinin (CTZ- εACA-BK) is a full agonist of the B_2R, but with a low affinity [9]. A similarly designed analog, carboxyfluorescein-εACA-BK, has an affinity for ACE that is identical to that of BK [8]. Testing the concept

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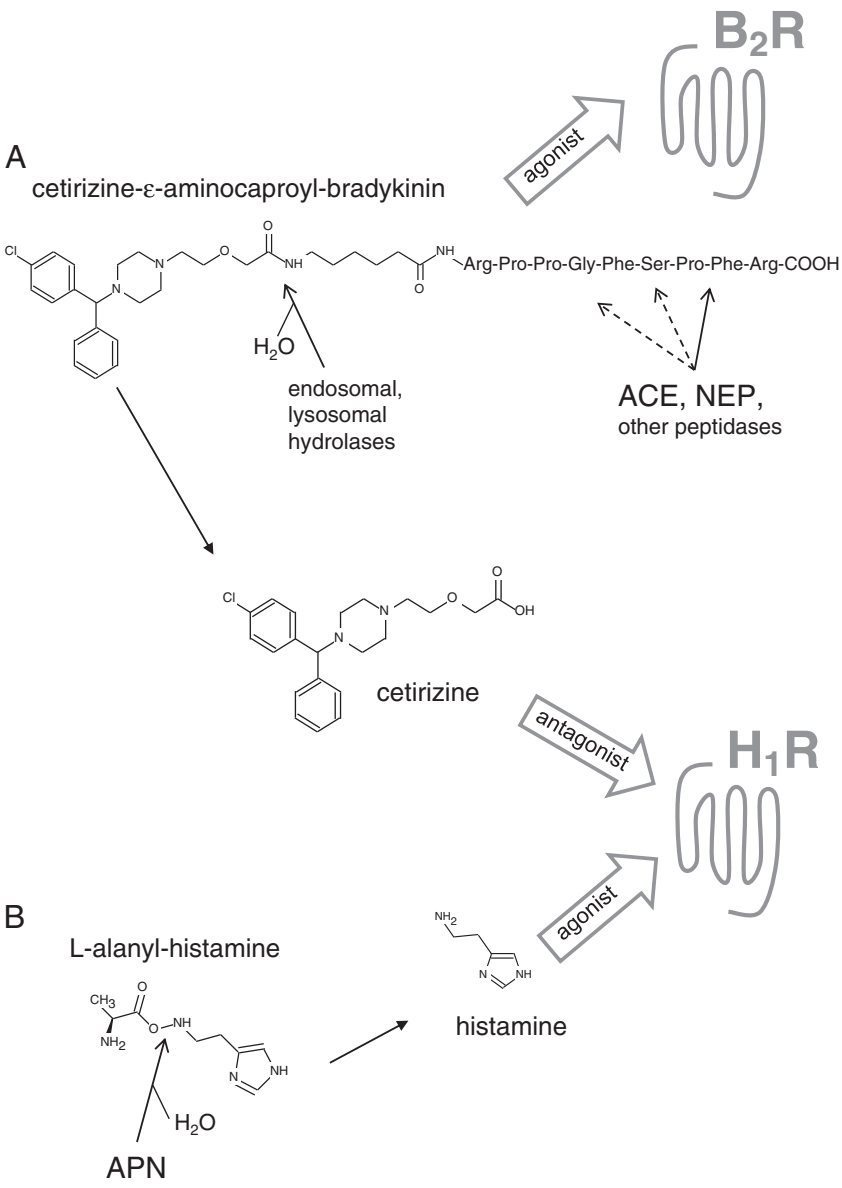

Fig. 1. Structure of the tested prodrugs and potential enzymatic reactions that may release H_1R ligands from them. A. Cetirizine-ε-aminocaproyl-BK (CTZ-εACA-BK), a known BK B_2R agonist. B. L-Alanyl-histamine. Some of the arrows indicate putative hydrolysis sites by enzymes (ACE: angiotensin converting enzyme; NEP: neutral endopeptidase; APN: aminopeptidase N).

of a pro-drug activable by vascular tissue metabolism may be based on CTZ-εACA-BK because cetirizine has a high persistence of its binding to histamine H_1 receptor (H_1R) [11]. Conjugated cetirizine, with its carboxylic acid function engaged in an amide bond, is predicted to have a low or no affinity for the H_1R. An alternative pro-drug potentially activated by a vasopeptidase was based on the agonist histamine condensed with alanine: L-alanyl-histamine (Fig. 1) was tested as a latent H_1R agonist activable by aminopeptidase N (APN, CD13, EC 3.4.11.2), on the model of the standard chromogenic substrate of APN, L-Ala-p-nitroanilide [12]. In addition to molecular studies based on recombinant B_2R and H_1R, we exploited the human umbilical vein contractility assay naturally expressing these two receptor types that mediate important contractile responses [13,14]. This vein also expresses ACE [2,15]. The ectopeptidase APN is present in the vascular smooth muscle cells of the umbilical artery [3,4] and at least in the endothelium of the umbilical vein [16,17].

2. Materials and methods

2.1. Synthesis of L-alanyl-histamine

The synthesis of L-alanyl-histamine (4-[β-(α-alanylamido)-ethyl]-1-*H*-imidazole (2-amino-N-[2-(1*H*-imidazol-4-yl)ethyl]-(2S)-propanamide) has been previously reported [18] based on Boc-L-Ala and tritylsulfenyl-histamine using the diisopropyl-carbodiimide coupling reagent. This method was slightly modified using histamine dihydrochloride and the BOP coupling reagent. Diisopropylethylamine (4 mmol, 697 μl) was added to the stirred suspension of Boc-Ala (1 mmol, 189.2 mg) (Benzotriazol-1-yloxy)tris(dimethylamino) phosphonium hexafluorophosphate (BOP, 1 mmol, 442.3 mg), and histamine dihydrocloride (1 mmol, 184.1 mg) in 25 ml acetonitrile. The mixture was stirred overnight at room temperature. The solvent was removed in vacuum and the oily residue was dissolved in 75 ml ethyl