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Short communication

The bradykinin B_2 receptor antagonist icatibant (Hoe 140) blocks aminopeptidase N at micromolar concentrations: Off-target alterations of signaling mediated by the bradykinin B_1 and angiotensin receptors

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Abstract

The N-terminal sequence of icatibant, a widely used peptide antagonist of the bradykinin B_2 receptors, is analogous to that of other known aminopeptidase N inhibitors. Icatibant competitively inhibited the hydrolysis of L-Ala-*p*-nitroanilide by recombinant aminopeptidase N (K_i 9.1 μM). In the rabbit aorta, icatibant (10–30 μM) potentiated angiotensin III, but not angiotensin II (contraction mediated by angiotensin AT_1 receptors), and Lys-des-Arg^9-bradykinin, but not des-Arg^9-bradykinin (effects mediated by the bradykinin B_1 receptors), consistent with the known susceptibility of these agonists to aminopeptidase N. At concentrations possibly reached in vivo (e.g., in kidneys), icatibant alters physiological systems different from bradykinin B_2 receptors.
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1. Introduction

Icatibant (Hoe 140; D-Arg[Hyp^3, Thi^5, D-Tic^7, Oic^8]-bradykinin) is a "second generation" peptide antagonist of the bradykinin B_2 receptor with nanomolar affinity (Leeb-Lundberg et al., 2005; Fortin and Marceau, 2006). This compound has been widely used as a tool to dissect the role of the kallikrein–kinin system in physiology and pathology, as it has been exploited in hundreds of research papers and more than 15 clinical studies; further, it is in clinical trial for hereditary angioedema and ascites associated with liver cirrhosis (Rosenkranz et al., 2005). Icatibant is a sequence-related, high affinity and peptidase-resistant antagonist of bradykinin with variable properties from one mammalian species to another. Thus, it is fully surmountable at the human bradykinin B_2 receptor, but insurmountable and practically irreversible at the rabbit orthologue; it is a partial agonist at the sheep receptor and a full agonist at the ornithokinin receptor, the chicken protein most related to the mammalian bradykinin B_2 receptor (Leeb-Lundberg et al., 2005).

We have lately discovered that icatibant and several other bradykinin receptor ligands can act as micromolar affinity inhibitors of crude aminopeptidase N (CD13), a widely distributed ectoenzyme capable of degrading various vasoactive peptides related or not to bradykinin (Gera et al., 2006). Aminopeptidase N is an important inactivation pathway for the optimal agonist of the human and rabbit bradykinin B_1 receptor, Lys-des-Arg^9-bradykinin (des-Arg^{10}-kallidin) (Pelorosso et al.,

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2005; Fortin et al., 2005; Gera et al., 2006). In addition, as a polyvalent member of the "degradome", it inactivates some neuropeptides, interleukin-8 (Riemann et al., 1999) and angiotensin III, a peptide believed to mediate a pressor effect in the brain and kidneys (Reaux et al., 2001; Farjah et al., 2004). Thus, estimating the in vivo role of the bradykinin B_2 receptor using icatibant, especially its vasodilator and salutary effects in hypertension, may be misleading because off-target effects may include the potentiation of a pressor peptide (angiotensin III) and of an inflammatory mediator (Lys-des-Arg^9-bradykinin) capable of increasing the sympathetic activity and peripheral vascular resistance when protected from peptidases (Audet et al., 1997). Numerous nonpeptide antagonists of both bradykinin receptor subtypes have been developed in recent years (Fortin and Marceau, 2006). These drugs are more conventional as far as biotransport is concerned, and perhaps less prone to off-target actions. LF 16-0687 (anatibant) is an excellent example of a relatively water soluble competitive antagonist of both the human and rabbit bradykinin B_2 receptors (Pruneau et al., 1999; Houle et al., 2000).

The present study aims to verify whether icatibant can exert physiological off-target effects due to the potentiation of angiotensin III and Lys-des-Arg^9-bradykinin in the isolated rabbit aorta; these effects are mediated respectively by the angiotensin AT_1 and bradykinin B_1 receptors, but modulated by the expression of aminopeptidase N in smooth muscle layers of this tissue (Fortin et al., 2005; Gera et al., 2006). The relevant molecular interaction of icatibant has been quantified and characterized using recombinant human aminopeptidase N (Breslin et al., 2003). A modern nonpeptide bradykinin B_2 receptor antagonist, LF 16-0687 (anatibant), has been compared with icatibant in these assays in an attempt to clarify the pharmacological specificity of alternate probes for the kallikrein–kinin system.

2. Materials and methods

2.1. Drugs

The nonpeptide LF 16.0687 (anatibant; 1-[[2,4-dichloro-3-[(2,4-dimethylquinolin-8-yl)oxy]methyl]phenyl]sulfonyl]-*N*-[3-[[4-(aminoiminomethyl]-phenyl]carbonylamino]propyl]-2(*S*)-pyrrolidinecarboxamide, mesylate salt) and the peptide icatibant (Hoe 140), antagonists of bradykinin at the rabbit and human bradykinin B_2 receptors (Pruneau et al., 1999; Houle et al., 2000), were gifts from Dr. Didier Pruneau, Laboratoires Fournier (Daix, France).

2.2. Enzymatic assay

Enzymatic assays, based on the chromogenic substrate L-alanine-*p*-nitroanilide (L-Ala-pNA, Sigma-Aldrich; 0.15–2.5 mM) and human recombinant aminopeptidase N (Breslin et al., 2003) (14 ng of protein per tube) co-incubated at 37 °C for 1–2 h in 200 µl of PBS, pH 7.4, were performed precisely as described (200 µl reaction volume) (Lendeckel et al., 1996). Colorless solutions of ligands of the bradykinin B_2 receptors (icatibant or LF 16-0687, 10–25 µM) were used as competitors of L-Ala-pNA hydrolysis in some experiments. Michaelis–Menten enzyme kinetics and competitive inhibition K_i values were evaluated using a computer program (Tallarida and Murray, 1987).

2.3. Contractility studies

A local animal care committee approved the procedures based on rabbits. Rabbit aortic rings with intact endothelium were suspended under a tension of 2 g in 5 ml tissue baths containing oxygenated (95% O_2: 5% CO_2) and warmed (37 °C) Krebs solution (Fortin et al., 2005; Gera et al., 2006). The procedures described in our recent report (Gera et al., 2006) were precisely applied to measure the potency of angiotensin II and III in the presence or absence of a putative aminopeptidase N inhibitor (icatibant, applied 30 min before). Other tissues were stimulated with the bradykinin B_1 receptor agonists Lys-des-Arg^9-bradykinin or des-Arg^9-bradykinin, ligands of high and low affinity, respectively, in the presence or absence of icatibant or LF 16-0687 (applied 30 min before). The concentration–response curves for the contraction induced by the bradykinin B_1 receptor agonists were constructed 5.5 h from tissue mounting (Fortin et al., 2005).

3. Results

3.1. Enzyme assays

Human recombinant aminopeptidase N hydrolyzed L-Ala-pNA with an apparent K_M of 0.29 mM (Fig. 1A). Icatibant (25 µM, Fig. 1; 10 µM, not shown) acted as an apparently

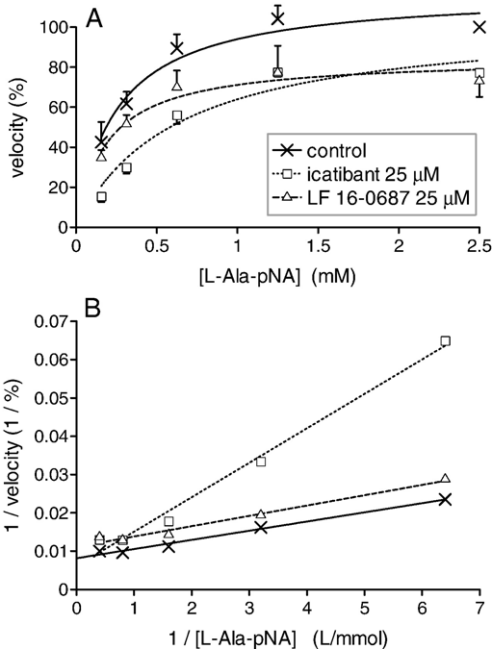

Fig. 1. Effect of two bradykinin B_2 receptor antagonists on the hydrolysis of L-Ala-pNA by human recombinant aminopeptidase N. A. Substrate hydrolysis as modified by icatibant of LF 16-0687 (25 µM of each). Results (mean ± s.e.m., $n=4$) are normalized as a percentage of the control velocity recorded with the 2.5 mM substrate concentration in each experiment (average 2.9 pkat). B. Double-reciprocal plot representation of the data from A.

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