Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 6, pp. 781-784, 1994

0960-894X/94 $6.00+0.00

0960-894X(94)E0050-O

p-Guanidinobenzoyl-[Hyp^3, Thi^5, D-Tic^7, Oic^8]bradykinin is Almost Completely Devoid of the Agonist Effect of HOE140 on the Endothelium-free Femoral Artery of Sheep.

Christophe Thurieau*, Michel Félétou, Emanuel Canet, Jean-Luc Fauchère.
Institut de Recherches Servier, 11, rue des Moulineaux, 92150, Suresnes, France.

Abstract. Substitution of the N-terminal arginine of [D-Arg^0, Hyp^3, Thi^5, D-Tic^7, Oic^8]bradykinin (HOE140) by 4-guanidinobenzoic acid results in an analogue with at least the same antagonistic potency on rabbit smooth muscle preparations but with considerable reduction of its agonistic properties on the endothelium-free femoral artery of sheep.

Bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) is an endogenous nonapeptide with a wide range of actions. In addition to its algesic[1] and proinflammatory effects[2], bradykinin induces endothelium-dependent vasodilatation, and vascular and bronchial smooth muscle contraction[3]. The actions of bradykinin are mediated through at least two different types of receptors[4] designated B1 and B2, but most of the early inflammatory effects of bradykinin are mediated by the B2 receptor subtype. There is a considerable effort in the development of bradykinin B2 receptor antagonists as potential therapeutic agents One of the most potent among them is [D-Arg^0, Hyp^3, Thi^5, D-Tic^7, Oic^8]bradykinin (HOE140, compound 1) which antagonizes the action of bradykinin at the B2 receptor at the nanomolar range[5,6], but which possesses agonistic properties on certain tissue preparations[7]

We report here that the substitution of D-Arg^0 of HOE140 by 4-guanidinobenzoic acid (Figure1) acid results in an analogue, (designated compound 2) with the same potency to antagonize the bradykinin-induced contractions in the rabbit jugular vein but with considerable reduction of its agonistic properties, in particular in the endothelium-free femoral artery of sheep

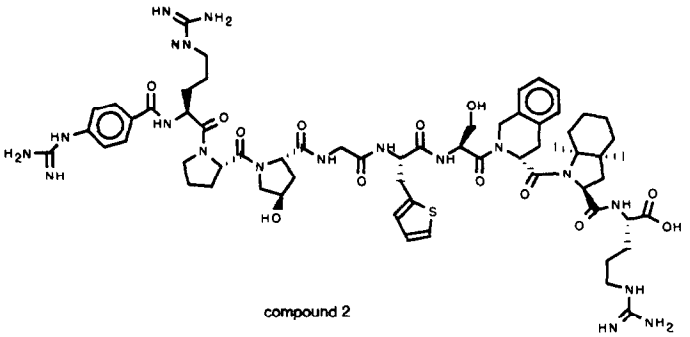

Figure1. Structure of p-Guanidinobenzoyl-[Hyp^3, Thi^5, D-Tic^7, Oic^8]bradykinin.

Materials and methods

Bradykinin and Des-Arg^9-bradykinin were from Sigma (La Verpillière, France). [D-Arg^0, Hyp^3, Thi^5, D-Tic^7, Oic^8]bradykinin (HOE140, compound 1) and p-Guanidinobenzoyl-[Hyp^3, Thi^5, D-Tic^7, Oic^8]bradykinin (compound 2) were synthesized by the solid phase method of Merrifield[8] using standard procedures on a Milligen 9050 peptide synthesizer The p-alkoxybenzyl alcohol resin, Nα-protected (Fmoc) aminoacids[9] were purchased from Bachem except for Fmoc-Oic-OH which was synthesized in house Single diisopropyl carbodiimide and N-hydroxy benzotriazole-mediated coupling was used except for D-Tic^7 and Pro^3 where TBTU and N-hydroxy benzotriazole were used as coupling reagents Peptides were cleaved from the resin by TFA (10ml/g of the resin) containing 30% of a dichloromethane/anisole/ethane dithiol (2/1/1, in ml/g of the resin) mixture Peptides were purified by preparative HPLC on a Waters Prep LC 3000 system equipped with a Waters 490E multiwavelength detector on a PrePak cartridge (47 x 300 mm) filled with a C18-Silica (300A, 15mm) phase The operating flow rate was 60 mL/min Purity was estimated to be >99% by analytical HPLC on a Waters 625 LC system equipped with a photodiode array UV detector, utilizing a DeltaPak C_{18} (spherical 5 μm) column (3.6 x 150mm). Amino acid analyses were performed with a Varian LC90 Star system and molecular weigths of peptides were determined by FAB mass spectrometry on a Normag R10-10C apparatus

Smooth muscle preparations were obtained as previously described[5,7] A reference contraction was produced with a concentration of KCl (60mM) which gave the maximum contraction to the depolarizing solution in those tissues Antagonists were allowed to equilibrate before the cumulative addition of agonists (i e bradykinin). Data were expressed in % of the reference contraction (KCl 60mM) pA2 values were calculated following Tallarida's method[10]

Table1 Antagonistic effects of bradykinin analogues

peptide	pA2 rabbit jugular vein (B_2)	a) rabbit aorta (B_1)
Compound 1	9 04 ± 0 28 (7)	IN
Compound 2	9 58 ± 0 01 (7)	IN

a) Lack of agonistic activity Data are shown as means ± SEM Numbers in brackets indicate the number of animals from which tissue was taken IN Inactive as antagonist

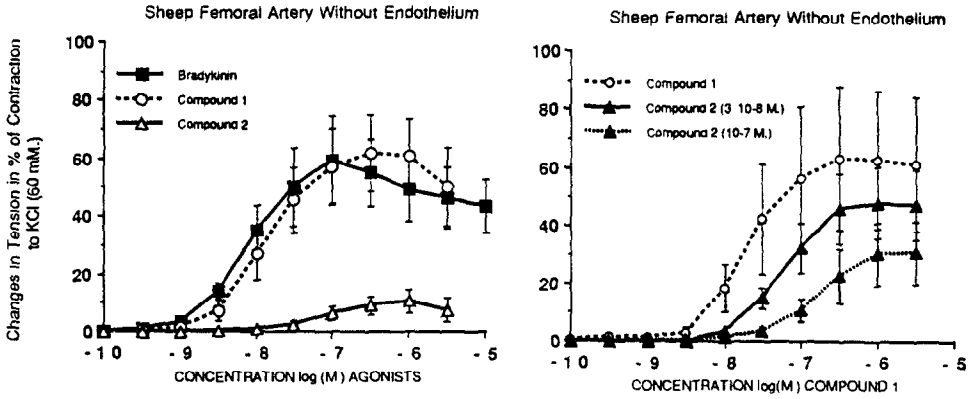

Figure 2. A: Agonistic effects of bradykinin analogues in isolated sheep femoral artery without endothelium Data are shown as means ± SEM
B: Antagonistic effects of compound 2 on the contractile response induced by compound 1 in the isolated sheep femoral artery without endothelium Data are shown as means ± SEM

Results and Discussion

The final peptide (compound 2, tris-trifluoroacetate, M=1651 6) had a single peak in HPLC (retention time 11min in isocratic conditions of 22% acetonitrile), the expected aminoacid content (±10%) and molecular weight (Fab-mass spectrometry: $[M+H]^+$=1309, M=1309 5 + trifluoroacetate counterions) Reference peptide (compound 1) was analyzed by the same method The antagonistic potencies on rabbit smooth muscle preparations of the two bradykinin analogues are presented in Table1. Compound 2 like compound 1 appears as a potent selective antagonist of the bradykinin B2 receptors (Rabbit Jugular Vein) with no antagonistic nor agonistic activity on the bradykinin B1 receptors (Rabbit Aorta). In the sheep femoral artery without endothelium (Figure 2A), HOE140 had the same efficacy (identical maximal effect) and very similar potency as bradykinin for this receptor In contrast, compound 2 had a very weak agonistic activity The contractions induced by HOE140 and bradykinin could be attributed to B2 receptor stimulation since compound 2 antagonized it but not Des-Arg^9-Leu^8 bradykinin (Figure 2B) Our results suggest that even minor changes in the structure of bradykinin analogues may be of importance to eliminate agonistic properties from B2-receptor antagonists. This is shown by the substantial decrease of agonistic activity of compound 2 compared to HOE140 from which it only differs in the replacement of a D-Arginine by a 4-guanidinobenzoic acid at the N-terminus of the molecule This substitution, which suppresses one asymmetric center, seems to significantly influence the interaction of the peptide with a subtype of

the B2-receptor and to decrease its agonistic potency, at least on the sheep femoral artery

In conclusion, the present study provides new information on the structural factors required for B2-antagonism and the new peptide describes herein can be considered as one of the most potent B2-receptor antagonist tested to date with minimal agonistic properties at least on the bradykinin-receptors subtype tested so far

Acknowledgments We wish to thank Christel Guyard and Hélène Carsalade for expert technical assistance

References and notes

Abbreviations used in this manuscript: B1= bradykinin receptor subtype 1, B2= bradykinin receptor subtype 2, Hyp = hydroxyproline, Oic = L-3as,7as-octahydroindole-2-carboxylic acid, Tic = 1,2,3,4-tetraisoquinoline-3-carboxylic acid, TBTU = 2-(1H-benzotriazol-1yl)-1,1,3,3-tetramethyl-uronium tetrafluoroborate, Thi = tetrahydro-1,4-thiazine-3-carboxylic acid, TFA = trifluoroacetic acid

1 Steranka, L R , Manning, D C , Dehaas, C J , Ferkany, J W , Borosky, S A , Connor, J R , Vavrek, R J , Stewart, J M , Snyder,S H *Proc.Natl.Acad.Sci.USA* **1988**,85,3245

2 Proud, D , Kaplan, A D *Annu.Rev.Immunol.* **1988**,6,49

3 Bhoola, K D , Figuerou, C D , Worthy, K. *Pharmacol. Rev.* **1992**, 44,1

4 Regoli, D., Barabe, *J. Pharmacol.Rev.* **1980**, 32, 1.

5 Hock, F G , Wirth, K , Albus, U , Linz, W ; Gerhards, H J ; Wiemer, G , Henke, St.; Breipohl, G , Konig, W , Knolle, J , Scholkens, B A. *Br.J.Pharmacol.* **1991**, 102, 769

6. Wirth, K , Hock, F G , Albus, U., Linz, W , Alperman, H G , Anagnostopoulos, H., Henke, St , Breipohl, G , Konig, W , Knolle, J , Scholkens, B.A *Br.J.Pharmacol.* **1991**, 102, 774

7 Félétou, M , Germain, M., Thurieau, C ; Fauchère, J-L., Canet, E **1994**, *Can. J. Physiol. Pharm.*, in press

8 Barany, G , Merrifield, R B *The Peptides, Analysis, Synthesis and Biology* Gross, E., Meienhofer, J , Eds, Academic Press New York,1979, 2, pp 3-284

9 Carpino, L A ; Han, G Y *J.Org.Chem.* **1972**, 37, 3404

10 Tallarida, R.J , Couvan, A , Adler, M W *Life Sci.* **1979**, 25, 637

(*Received in Belgium* 17 *December* 1993; *accepted* 31 *January* 1994)