Elucidation of the Structure-Activity Relationships of Apelin: Influence of Unnatural Amino Acids on Binding, Signaling and Plasma Stability

Alexandre Murza[1,2], Alexandre Parent[2], Elie Besserer-Offroy[1,2], Nicolas Beaudet[2], Philippe Sarret[2], Eric Marsault[1]

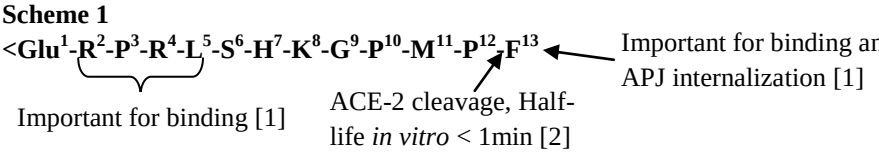
[1]*Department of Pharmacology,* [2]*Department of Physiology and Biophysics, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, QC, Canada*

eric.marsault@usherbrooke.ca

Introduction

Apelin is the endogenous ligand of APJ receptor, a member of the G protein-coupled receptor superfamily. There is currently little information on the structure/activity relationship (SAR) of apelin (**Scheme 1**). In an effort to better delineate SAR, we synthesized analogs of apelin-13 modified at selected positions with unnatural amino acids, with a particular emphasis on the C-terminal portion and Pro[12]. Analogs were then tested in binding and functional assays by evaluating $G_{i/o}$ mediated reduction in cAMP levels and by assessing β-arrestin2 recruitment to the receptor. The plasma stability of new analogs was also assessed. Several were found to possess increased binding, biased β-arrestin2 signaling and higher stability compared to the parent peptide.

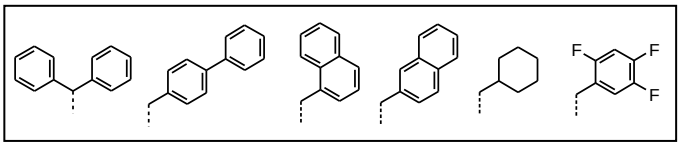
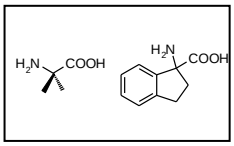
Scheme 1



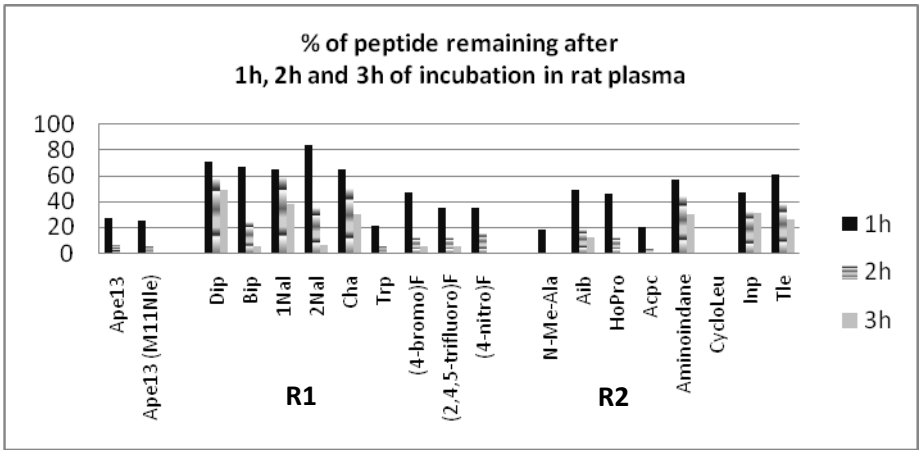
Results and Discussion

The C-terminal Phe[13] of apelin-13 was replaced by unnatural amino acids (**R1**, **Table 1**). This set of modifications was performed on the Met11Nle analog which possesses a similar profile in terms of affinity, coupling to second messenger cascades, and stability to that of apelin-13 (IC_{50} 5.7 nM ; $EC_{50\ cAMP}$ 1.9 nM ; $EC_{50\ \beta\text{-arr2}}$ 91 nM). Analogs Phe13Dip and Phe13Bip displayed a 10-fold difference in affinity suggesting that the C-terminal binding site is deep rather than wide. Interestingly, Phe13Cha exhibited an affinity comparable to that of apelin-13, indicating that hydrophobic interactions are necessary for binding, but aromatic, π -stacking type interactions are not essential. Phe13-1Nal and Phe13-2Nal showed an interesting trend in the β-arrestin2 pathway. Replacement of Pro[12] by Aib provided a very potent analog, and Pro12Aminoindane exhibited a biased signaling in β-arrestin2 pathway (**R2**, **Table 1**). Finally, C-terminally modified analogs showed significant improvements in plasma stability over apelin-13, whereas modification of Pro12 displayed more variable results (**Scheme 2**).

Table 1

	R1						R2	
								
	Dip	**Bip**	**1Nal**	**2Nal**	**Cha**	**(2,4,5-trifluoro)F**	**Aib**	**Aminoindane**
IC_{50} (nM)	**88 ± 6**	**7.8 ± 0.4**	14 ± 0.9	1.2 ± 0.1	**2.3 ± 0.6**	**0.8 ± 0.2**	**0.7 ± 0.1**	20 ± 1
EC_{50} cAMP (nM)	ND	10 ± 3	28 ± 2	20 ± 6	20 ± 8	20 ± 9	30 ± 13	13 ± 4
EC_{50} β-arr2 (nM)	630 ± 179	361 ± 64	**522 ± 110**	**70 ± 11**	170 ± 32	32 ± 9	46 ± 10	**1204 ± 208**

Scheme 2



Acknowledgments

Financial support from the Université de Sherbrooke, the Institut de Pharmacologie de Sherbrooke (IPS) and the Centre des Neurosciences de Sherbrooke (CNS).

References

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