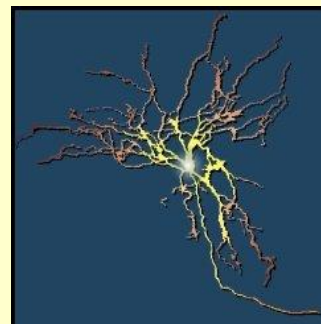
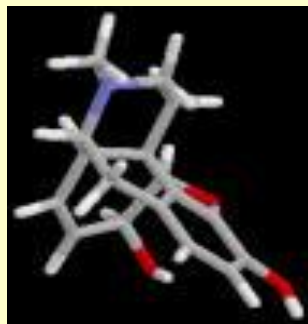


Szemezgetés a neuropeptidek köréből: opioid peptidek

Borsodi Anna
MTA Szegedi Biológiai Kutatóközpont



Ünnepi program az MTA-ELTE Peptidkémiai Kutatócsoport
megalakulásának 50. évfordulója alkalmából

Budapest 2011 dec. 9

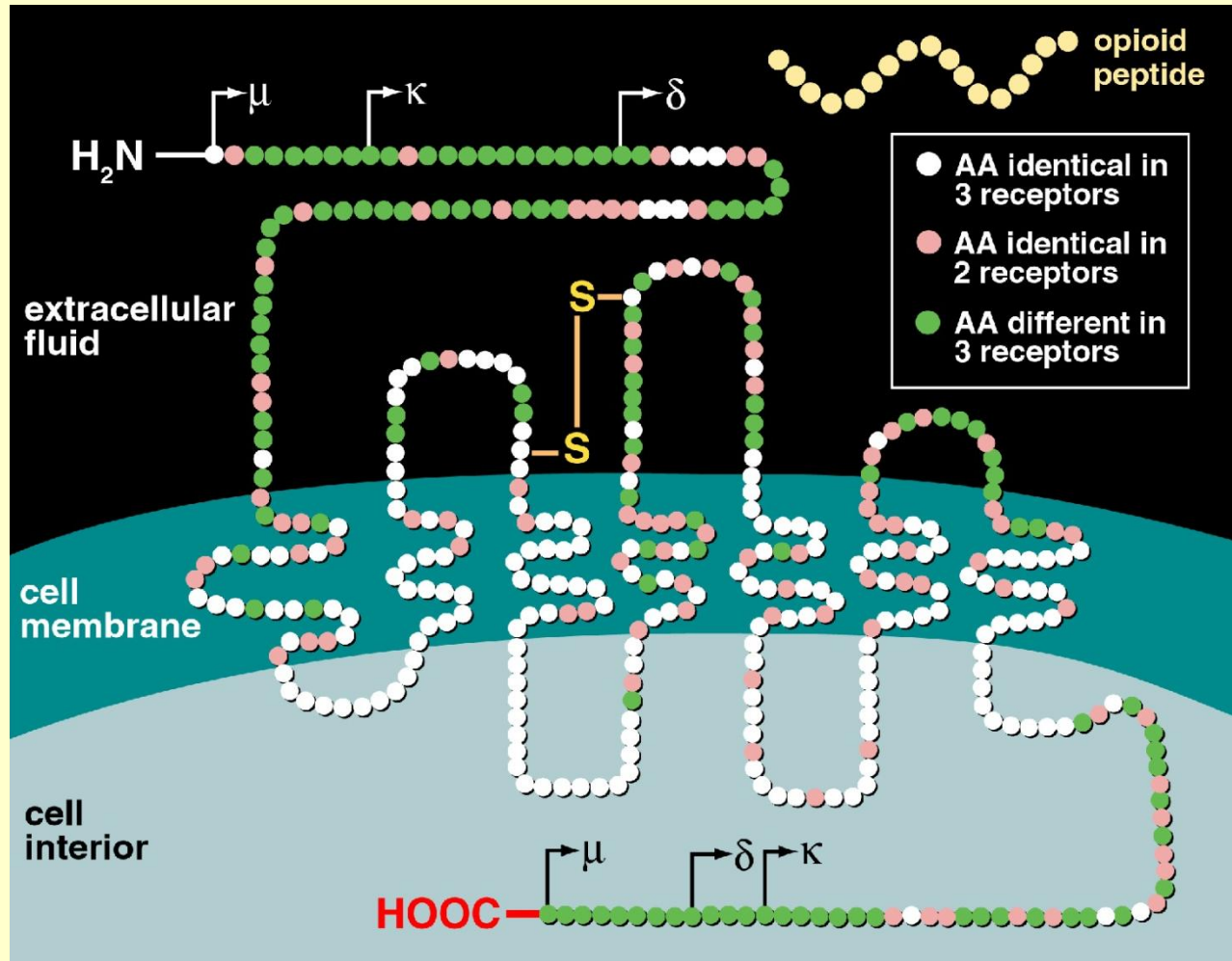


Wollemann Mária



MTA SZBK Szeged

Opioid receptorok elsődleges szerkezete: az emberi μ -, δ - és κ -receptor



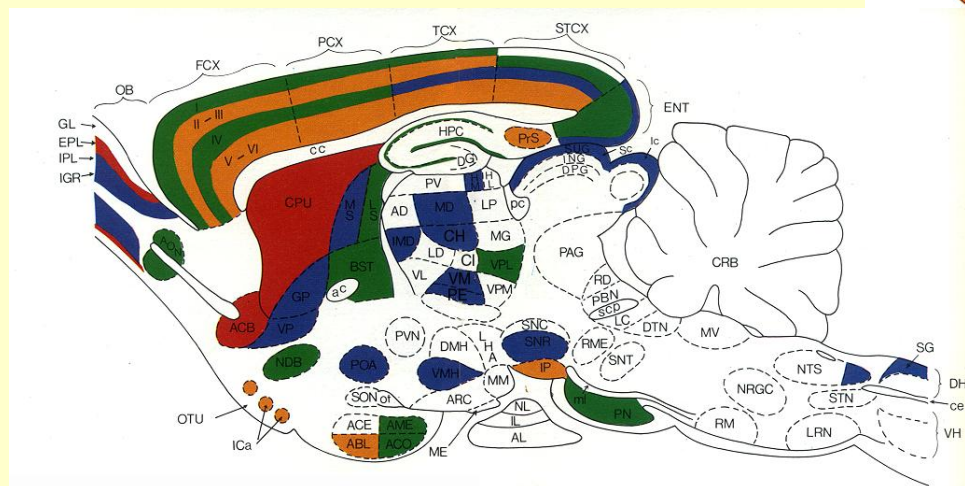
1973 (felfedezés) - 1992 (klónozás)

RECEPTOR HETEROGENITÁS

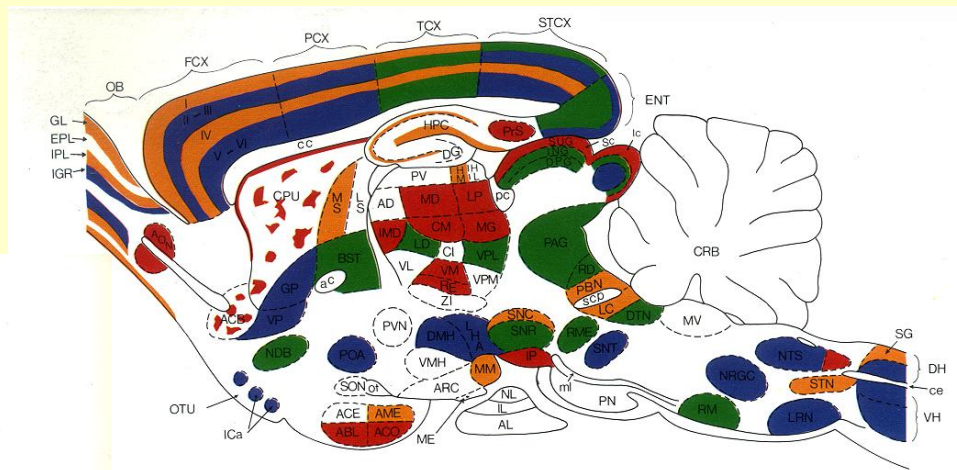
MU, DELTA, KAPPA

- FEHÉRJE SZERKEZET
- ANATÓMIAI LOKALIZÁCIÓ
- LIGAND SPECIFITÁS
- FIZIOLÓGIAI HATÁSOK

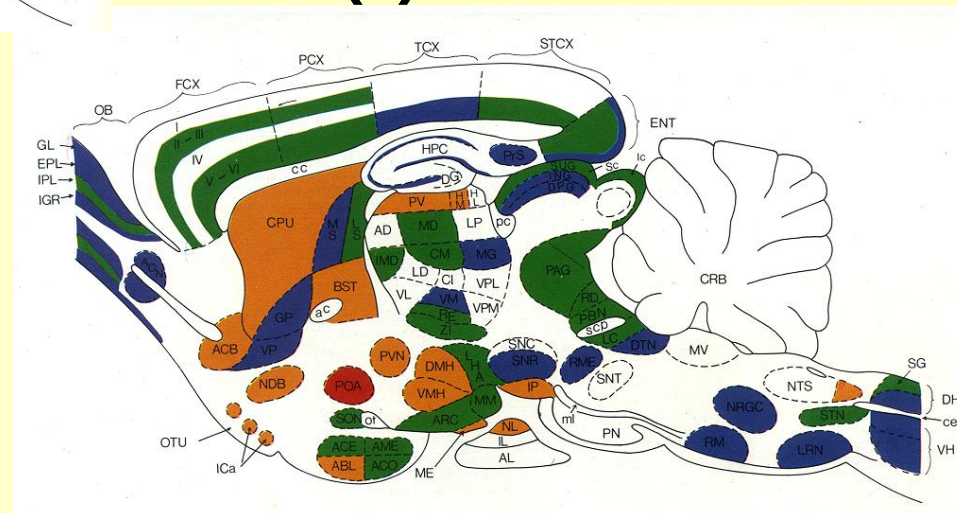
DOPr (δ)



MOPr (μ)



KOPr (κ)

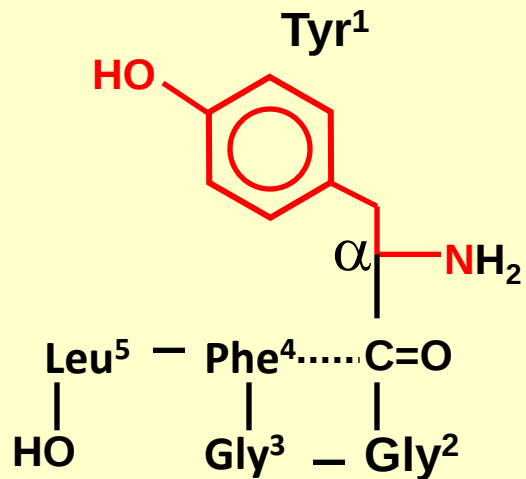


**Opioid receptorok
regionális
megoszlása
patkány agyban**

(Mansour et al., Trends NeuroSci, 1988)

Morphine and enkephalins: schematic structural comparisons

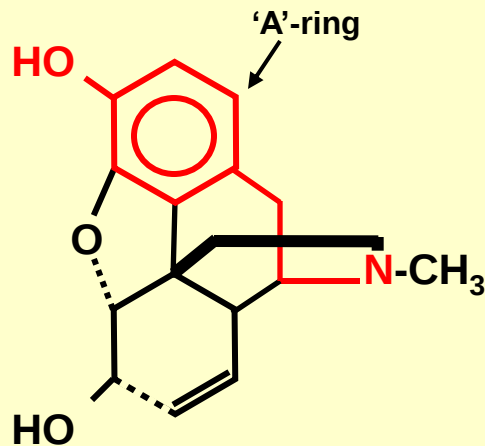
Leu-enkephalin



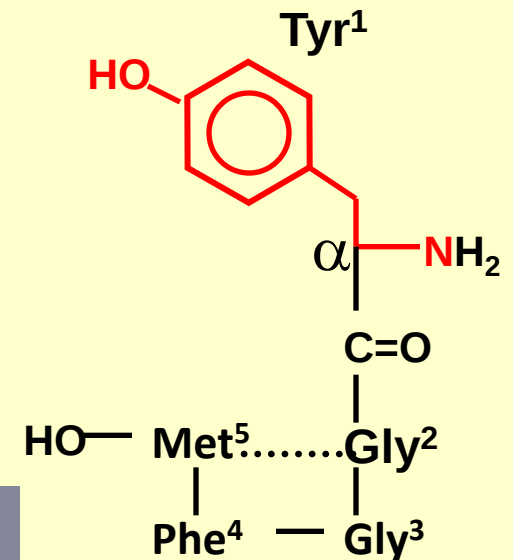
YGGFL

1-4 β -bend

Morphine

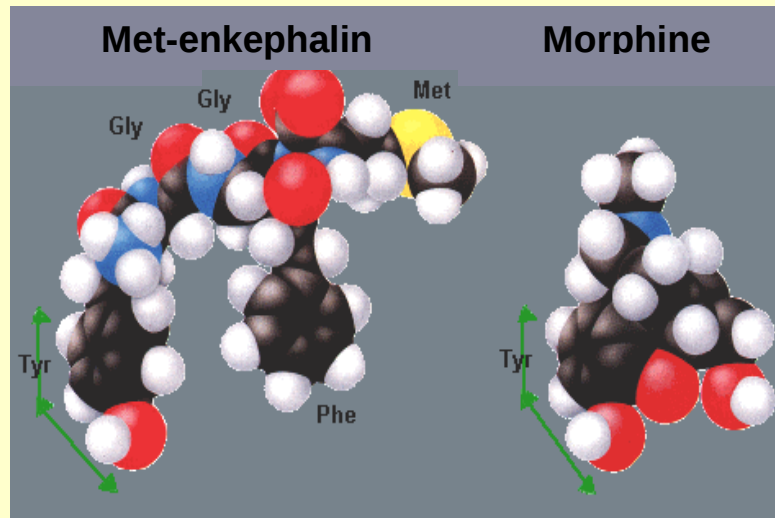


Met-enkephalin



YGGFM

2-5 β -bend



Receptor fehérje affinitásjelölése opioid peptidekkel

**Receptor fehérje tisztítás
affinitáskromatográfiával béka agyból**

Enkefalin klórmetil ketonok

Tyr - Gly -Gly- Phe - Leu-OH	Leu-enkefalin
Tyr - Gly -Gly- Phe - Leu-CH ₂ Cl	LECK
Tyr -D-Ala-Gly- Phe - Leu-CH ₂ Cl	DALECK
Tyr -D-Ala-Gly-NMePhe-Gly-ol	DAMGO
Tyr -D-Ala-Gly-NMePhe-Gly-CH ₂ Cl	DAMK

Species difference in ^3H -DAMCK labelling pattern

SPECIES/TISSUE	SUBUNIT OF	MOLECULAR LABELLED PROTEINS	WEIGHT (kDa)
----------------	---------------	--------------------------------	------------------

Rat / Forebrain	<u>58</u> ,	48, <u>38</u> ,	18
-----------------	-------------	-----------------	----

Rabbit / Cerebellum	<u>58</u> ,	48, <u>38</u> ,	18
---------------------	-------------	-----------------	----

Guineapig/ Cerebellum	<u>58</u> ,	48, <u>38</u> ,	18
-----------------------	-------------	-----------------	----

Frog / Brain	<u>58</u> ,		18
--------------	-------------	--	----

Adult Chicken Brain	70, <u>58</u> ,	48	
---------------------	-----------------	----	--

Neonate Chicken Brain	<u>58</u> ,	48	
-----------------------	-------------	----	--

Irreverzibilitás



Tyr-D-Ala-Gly-Phe-Mel-Ome

Mel-D-Ala-Gly-Phe-Leu-OH



N-terminális tirozin nélkül
(az aktivitás megőrzése)

RECEPTOR IZOLÁLÁS

KAPPA SZEPARÁLÁS BÉKÁBÓL **(natív méret alapján)**

TISZTÍTÁS

1.) HIDROFÓB KROMATOGRÁFIA

2.) AFFINITÁS KROMATOGRÁFIA

A) DALE

B) DINORFIN



KOPr tisztítás homogenitásig (1990)

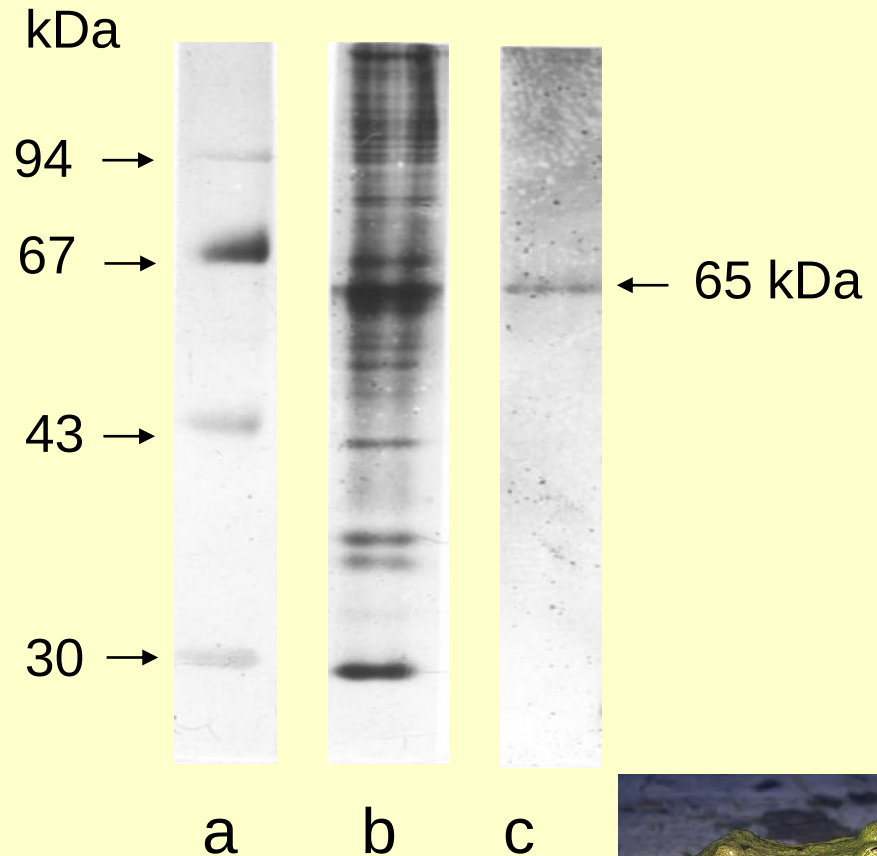
Membránpreparálás

Szolubilizálás

Affinitáskromatográfia

Ultraszűrés / koncentráció

Rekonstitúció lipidekkel



Kappa-opioid receptor: Purification from frog brain



JOURNAL OF
NEUROCHEMISTRY

OFFICIAL JOURNAL OF THE INTERNATIONAL SOCIETY FOR NEUROCHEMISTRY

Solubilization and Characterization of Opioid Binding Sites from Frog (*Rana esculenta*) Brain

J. Simon, M. Szücs, S. Benyhe, A. Borsodi, P. Zeman, and M. Wollemann

Neurochemical Research, Vol. 15, No. 9, 1990, pp. 899-904

NEUROCHEMICAL
RESEARCH

Characterization of Kappa₁ and Kappa₂ Opioid Binding Sites in Frog (*Rana esculenta*) Brain Membrane Preparation

Sandor Benyhe¹, Eva Varga¹, Jozsef Hepp², Anna Magyar², Anna Borsodi¹, and
Maria Wollemann¹

Volume 183, number 2

FEBS 2481



April 1985

Separation of κ -opioid receptor subtype from frog brain

József Simon, Sándor Benyhe, Anna Borsodi, Mária Szücs and Mária Wollemann

Journal of
Neuroscience
Research

Volume 56 Number 6 June 15, 1999

Journal of Neuroscience Research 25:549-555 (1990)

Method for Isolation of Kappa-Opioid Binding Sites by Dynorphin Affinity Chromatography

J. Simon, S. Benyhe, J. Hepp, E. Varga, K. Medzihradzsky, A. Borsodi,
and M. Wollemann

Journal of Neurochemistry
Lippincott-Raven Publishers, Philadelphia
© 1995 International Society for Neurochemistry

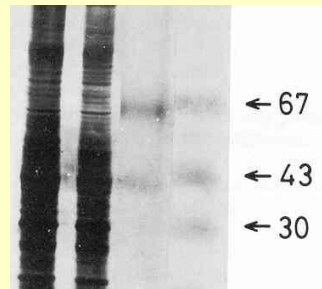


JOURNAL OF
NEUROCHEMISTRY

OFFICIAL JOURNAL OF THE INTERNATIONAL SOCIETY FOR NEUROCHEMISTRY

Thermodynamic Parameters of Frog Brain κ -Opioid Receptors

Jaideep Moitra, Huseyin Avni Öktem, and Anna Borsodi



OPIOID PEPTID TANDEM

**MTA-ELTE Peptidkémiai
Kutatócsoport**

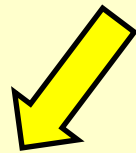
**MTA-SzBK Biokémiai Intézet
Opioid receptor csoportok**



LIGAND DESIGN

AIMS: to enhance - affinity, - specificity and - stability (peptides!)

LIGANDS



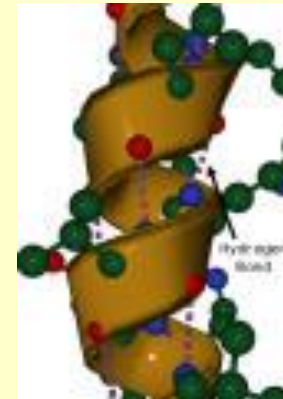
Heterocyclic compounds

Therapeutical applications



Peptides

Synthetic agonists/antagonists



Homology with opioid peptides

Orphanin FQ	-	F	G	G	F	T	G	A	R	K	S	A	R	K	L	A	N	Q
Dynorphin A	κ -	Y	G	G	F	L	R	R	I	R	P	K	L	K	W	D	N	Q
α -Endorphin	-	Y	G	G	F	M	T	S	E	K	S	Q	T	P	L	V	T	
Dynorphin B	-	Y	G	G	F	L	R	R	Q	F	K	V	V	T				
Leu-enkephalin	δ -	Y	G	G	F	L												
Endomorphin 1	μ -	Y	P	W	F													
Endomorphin 2	-	Y	P	F	F													

Morphine μ

“Message”

“Address”

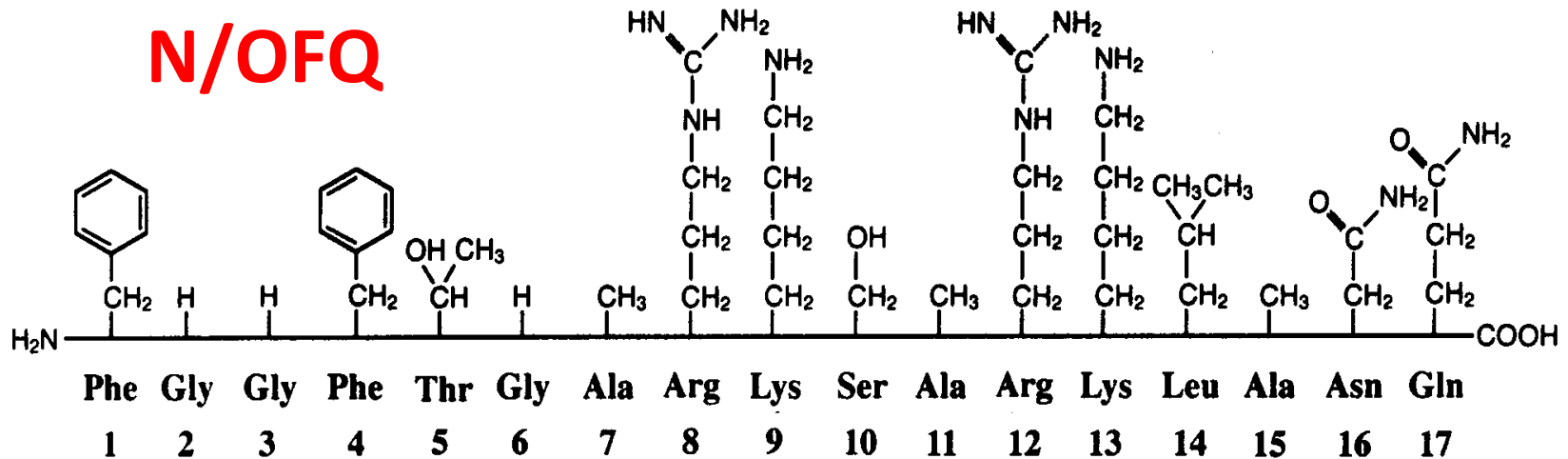
1997

Dooley *et al.* JPET

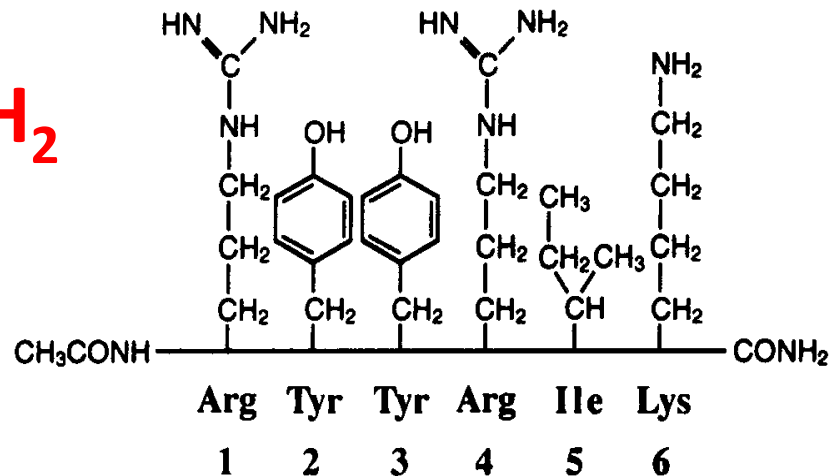
HEXAPEPTIDES

Ac-RYY-R/K-W/I-R/K-NH₂

N/OFG



Ac-RYYRIK-NH₂



Conclusions I - *in vitro* studies

Ac-RYYRIK-ol

✓ Receptor binding	<i>high affinity NOP ligand</i>	$pEC_{50} = 9.10$
✓ GTP γ S binding	<i>competitive antagonist</i>	$pA_2 = 8.67$
✓ mVD bioassay	<i>competitive antagonist</i>	$pA_2 = 8.46$
✓ mColon	<i>full agonist</i>	$pEC_{50} = 9.0$

- ✓ C-term reduction increases affinity
- ✓ Both N- and C-terminals are important
- ✓ Structural requirements for the hexapeptides;
 - Positively charged Arg¹ residue
 - N-acetyl group (protection)

Gunduz et al. 2006, *Eur. J. Pharmacol.* I
Gunduz et al. 2006, *NeuroSignals*,

Phylogenetic variability in proenkephalin- and prodynorphyn derived opioid peptides: an illustration of chemical biodiversity

Natural diversity of opioid peptides

**Genetically encoded
structure and biosynthesis**



Bioinformatic search of databases

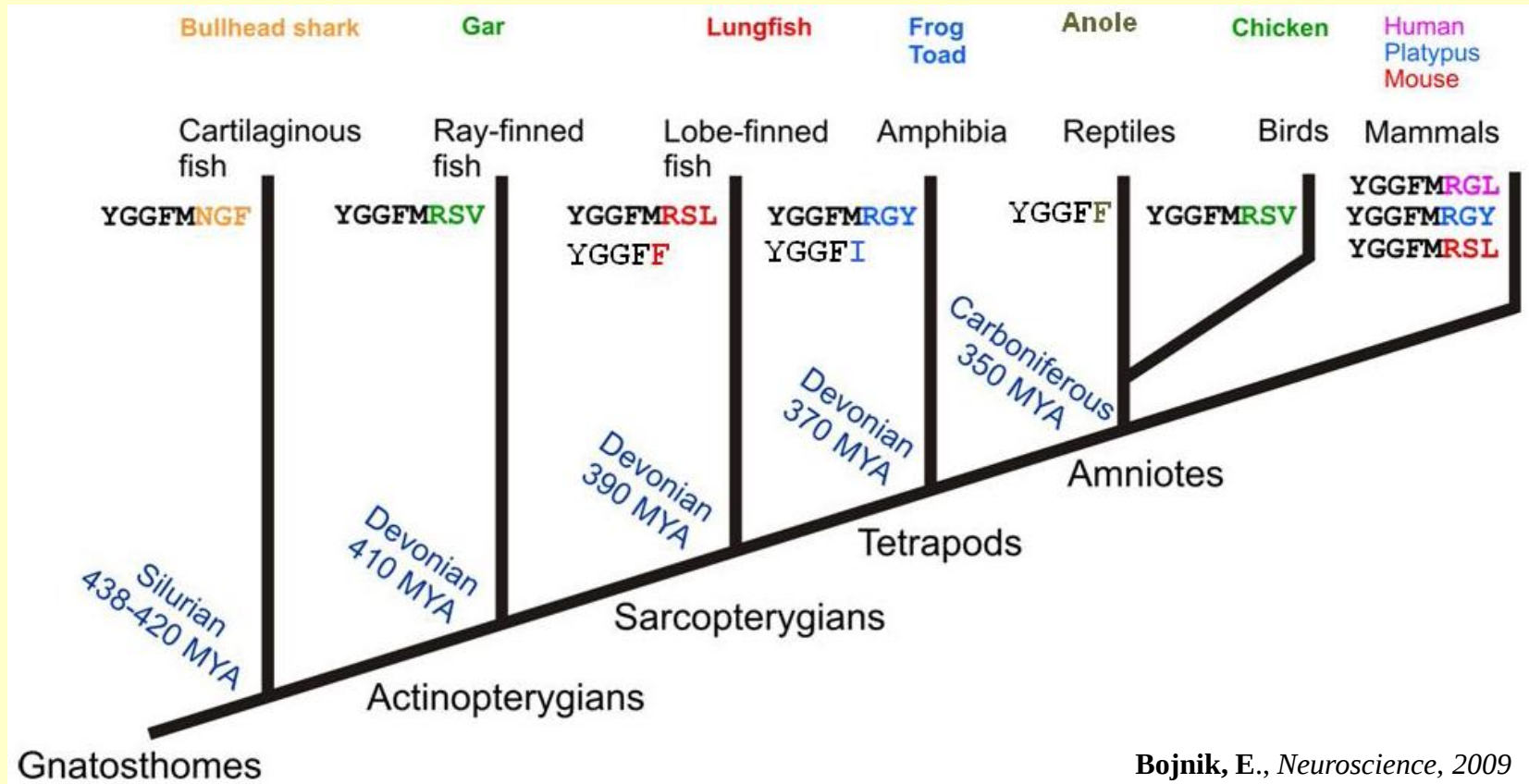


Chemical synthesis



Biochemical characterizations

Evolution scheme



YGGFI



YGGF**RGY**



YGGF**RSL**



YGGF**F**



YGGF**RSV**



YGGF**NGF**





Me

High Homology



My supervisor



A közös kutatások résztvevői

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**29 közös publikáció
1983-2010**

