Aula 12: 12 e 19/01/2021

Desenvolvimento de inibidores enzimáticos para uso terapêutico

(Ciência → Medicina, Farmácia & Saúde Pública Humana)

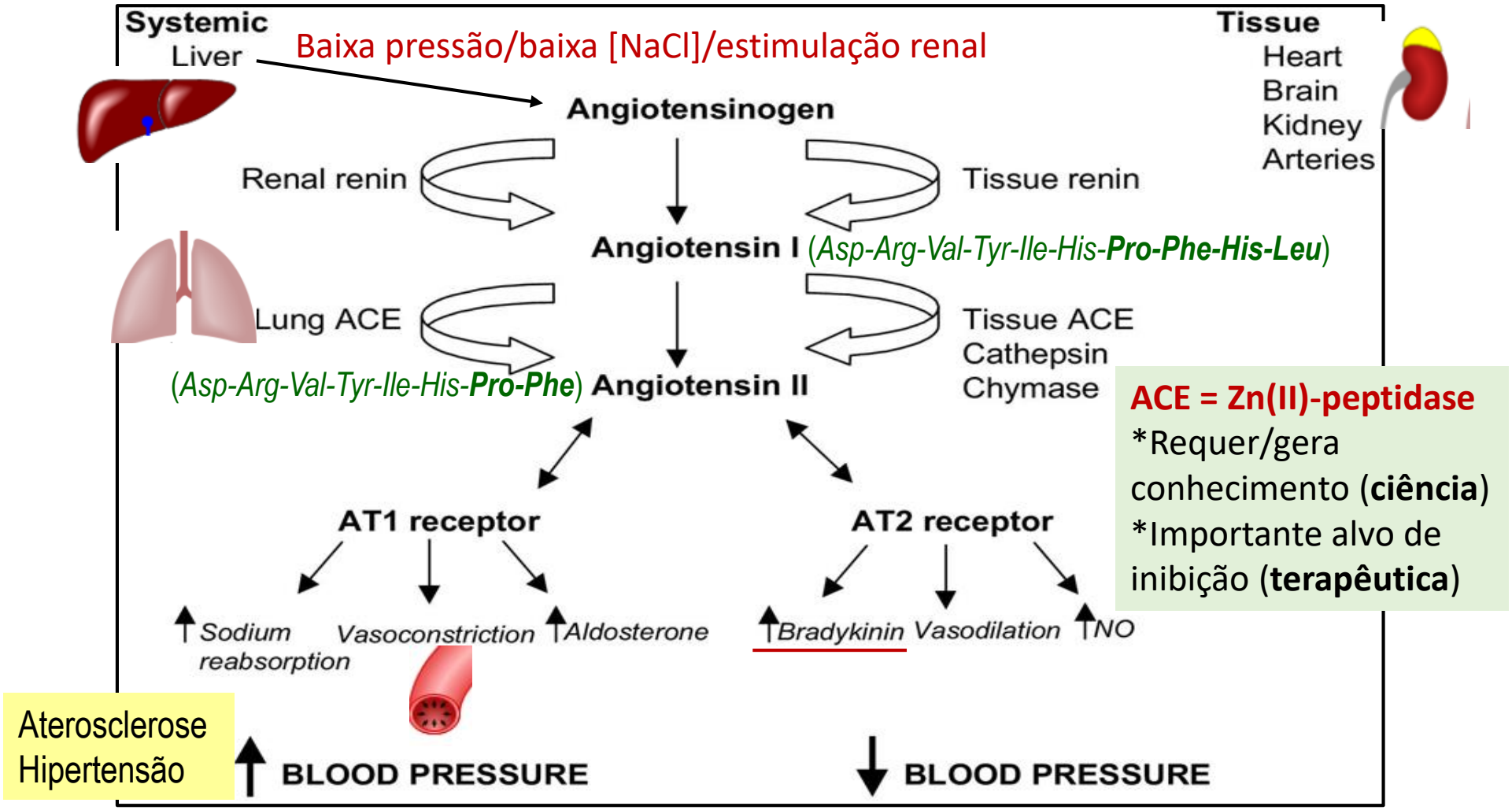
Fundamentos de aplicações atuais de enzimas em transformações químicas

(Ciência → Química, Biotecnologia & Saúde Ambiental)

vídeo #1

Sistema renina-angiotensina-aldosterona (SRAA) e bradicinina → controle da pressão arterial humana

(*década 1920*) (*anos 1940-60*)



ACE = Zn(II)-peptidase
*Requer/gera conhecimento (**ciência**)
*Importante alvo de inibição (**terapêutica**)

Aterosclerose
Hipertensão

Aldosterona (e a vasopressina): os rins retém sódio → água retida, aumento do volume de sangue e pressão arterial. Também faz com que os rins excretem potássio.
NO: relaxamento do músculo liso da parede dos vasos sanguíneos, dilatando-o.

Bradicinina (BK): peptídeo hipotensor

1949: **M. Rocha e Silva e cols, FMRP-USP**

plasma humano + veneno de *Bothrops jararaca* brasileira

Fator(es) hipotensor(es) e espasmogênico(s) da musculatura lisa

Cromatografias /Eletroforeses /Determinação de sequências

Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg

Síntese de BK → **A BK sintética foi menos ativa...**

...Devido a fatores potenciadores de BK

Brit. J. Pharmacol. (1965), **24**, 163–169.

A BRADYKININ-POTENTIATING FACTOR (BPF) PRESENT IN THE VENOM OF *BOTHROPS JARARACA*

BY

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↓

Menor deles → Glu-Lys-Trp-Ala-Pro

↓

Década de 70: nove peptídeos foram purificados e identificados

Teprotide: **Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro**

Mais estável à degradação enzimática: presença de 4 Pro

Alta atividade hipotensora

Caro

Inatividade oral

Juntando as informações com outras sobre membros da classe de enzimas de ACE ou ECA → → →

Zn(II)-containing proteases

JBC, 1967

CPA = carboxipeptidase

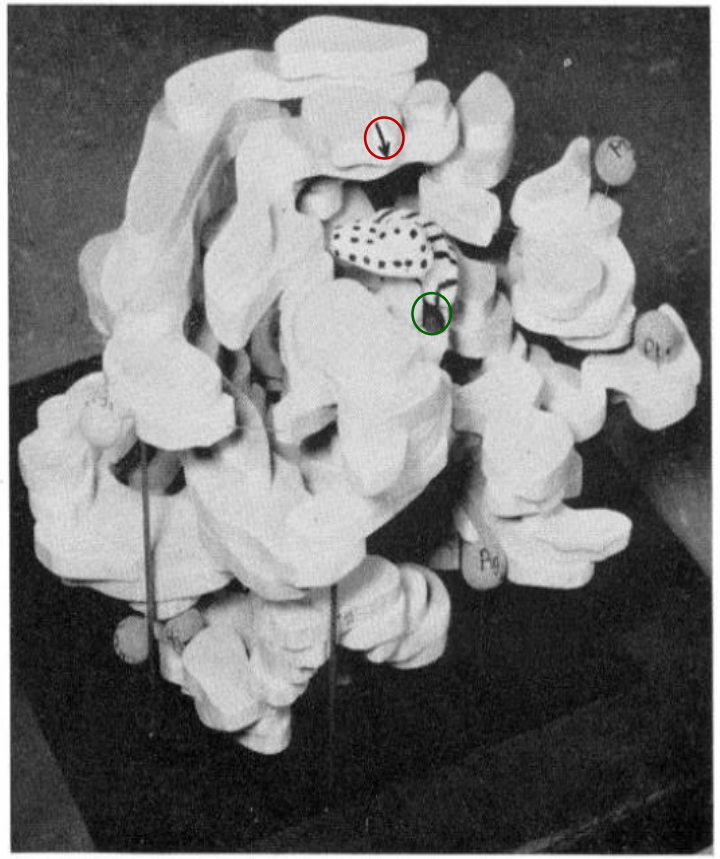
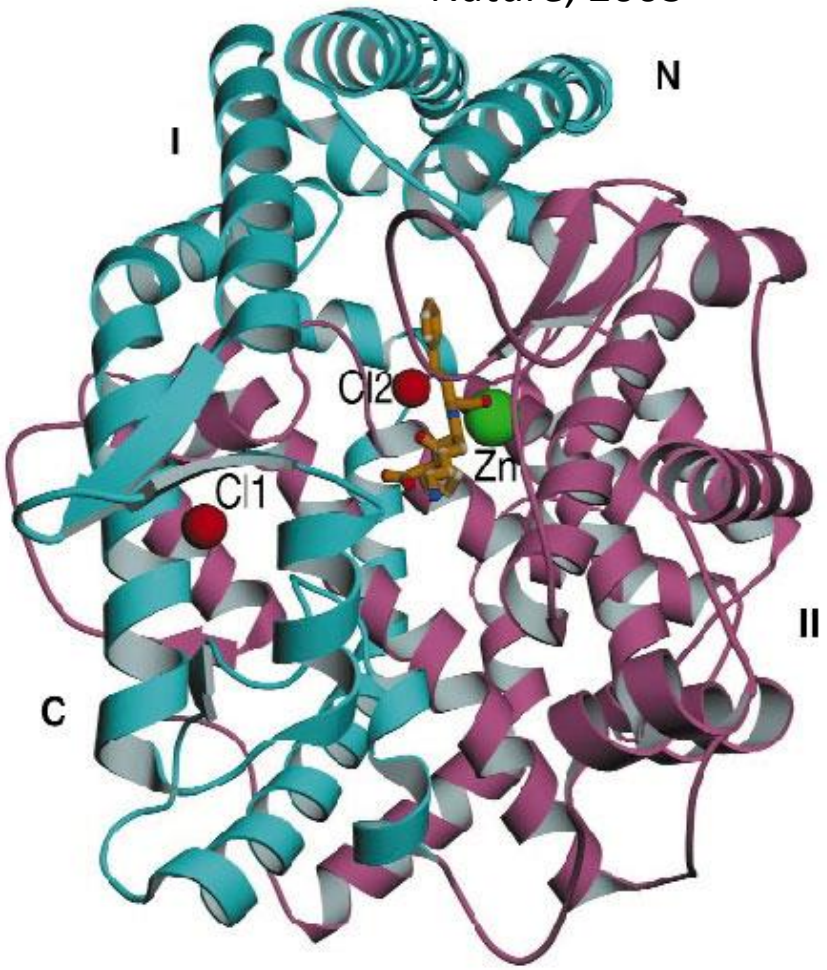


FIG. 5. Photograph of balsa model of 6 A CPA electron density map along with increase in electron density produced by 0.05 M glycyl-L-tyrosine. Region A is *striped* while Region B is *dotted*. The movement of protein which occurs when substrates are bound is indicated by the *arrow*. The *black spot* below Region A is the zinc atom. The crystallographic *b* axis is along the directions of the vertical support rods, while the *a* axis is along the front edge of the base.

Nature, 2003



Crystal structure of **human testicular ACE** with the inhibitor (***lisinopril***) molecule bound at the centre of the molecule. The green sphere represents the zinc ion and the red spheres represent the bound chloride ions.

Desenvolvimento de análogos sintéticos inibidores a partir de:

Menor BPP ativo obtido: Glu-Lys-Trp-Ala-Pro

Glu-Trp-Pro-Arg-Pro-Glu-Ile-Pro-Pro (Teprotide) (BPP_{9a}) I_{50} = 0.56 μM

Glu-Lys-Phe-Ala-Pro I_{50} = 0.9 μM

Phe-Ala-Pro I_{50} = 3.6 μM

Ala-Pro I_{50} = 230 μM

Resultante dos dados de análogos + outros inibidores peptídicos de outros venenos de cobra

Figure 1 Teprotide and analogues (Ondetti & Cushman, 1981).

Novos análogos...

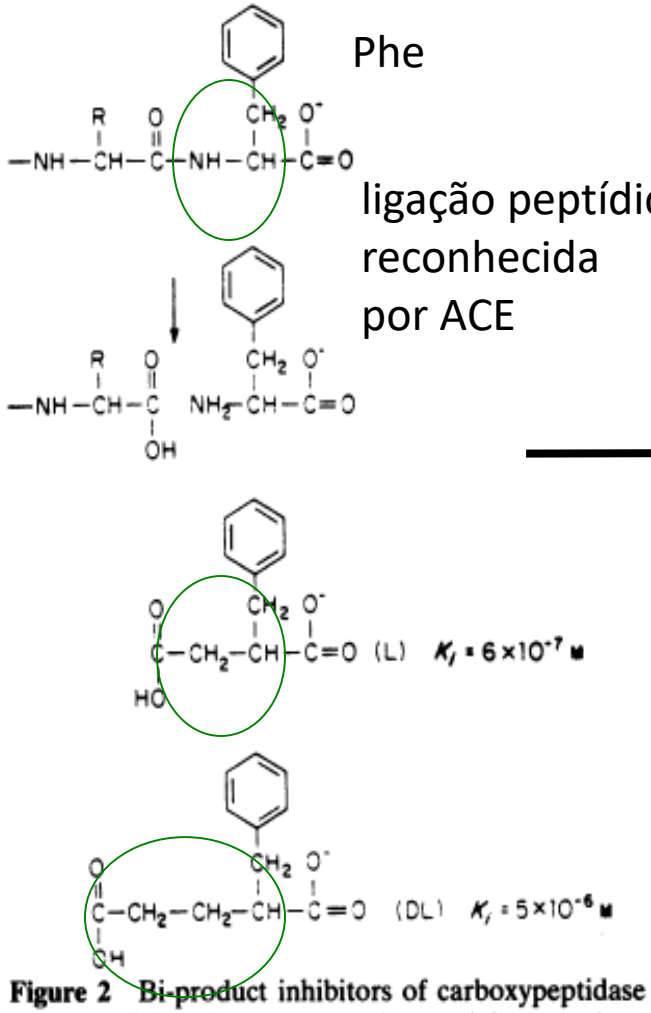


Figure 2 Bi-product inhibitors of carboxypeptidase A synthesised by Byers & Wolfenden (1972, 1973).

Table 1 Carboxyalkanoyl and mercaptoalkanoylproline inhibitors of angiotensin converting enzyme

dipeptídeo + carboxipeptidase

No.		Inhibition
1	(Pro) Succinil-Pro	43% at 3.3×10^{-4} M*
2		84% at 6.7×10^{-5} M*
3	(Ala)	I_{50} 2.2×10^{-5} M*
4		4.9×10^{-6} M*
5		2.3×10^{-8} M*

*The assay method is described by Patchett *et al.* (1980)

-SH liga Zn(II)!

Captopril-ACE ou ECA

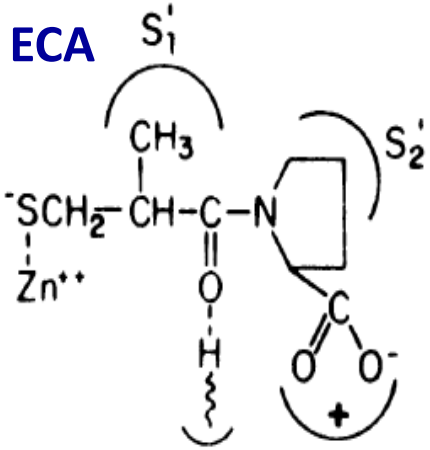


Figure 3 The proposed binding of captopril to angiotensin converting enzyme, adapted from Petrillo & Ondetti (1982).

Ki = 2 x 10^{-10} M / Boa atividade oral

SH susceptível à oxidação → S-S

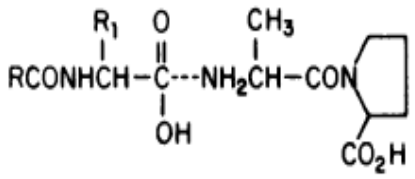
Since the 1980s, inhibitors of angiotensin converting enzyme (ACE inhibitors) have achieved great success as first-line therapy for cardiovascular diseases, including high blood pressure, heart failure, coronary artery disease, and kidney failure.

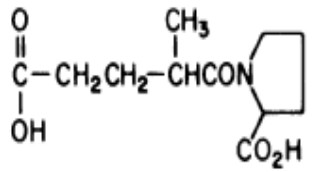
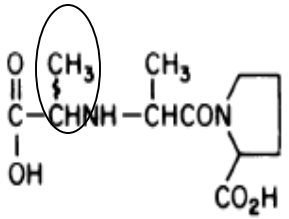
These anti-hypertensive drugs were not designed based on any knowledge of the three-dimensional structure of ACE, but on an assumed mechanistic homology to carboxypeptidase A, whose structure has been known for some time.

However, prolonged administration of current ACE inhibitors leads to several undesirable side effects, such as a persistent dry cough, headaches and dizziness.

Desenvolvimento do enalapril?

Table 2 The development of the N-carboxyalkyl dipeptides from a bi-product inhibitor design

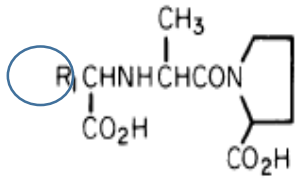


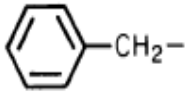
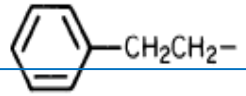
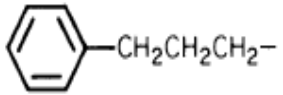
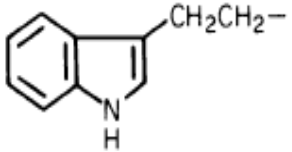
Number		I_{50}
4		4.9×10^{-6} M*
6		2.4×10^{-6} M†
7		9×10^{-8} M†

Maior interação com ACE→ Ala-Pro

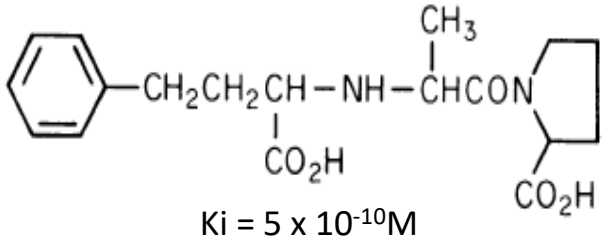
*Cushman *et al.* (1977).
†The assay method and the synthesis of these compounds are described by Patchett *et al.* (1980).

Table 3 Substituted carboxyalkyl derivatives of Ala-Pro



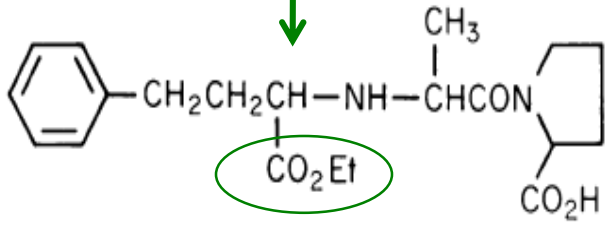
Number		I_{50}*
6	H–	2.4×10^{-6} M†
7	CH_3–	9×10^{-8} M†
8	CH_3CH_2–	1.7×10^{-8} M†
9	$CH_3CH_2CH_2$–	5×10^{-9} M‡
10	$(CH_3)_2CH$–	4×10^{-8} M‡
11	$(CH_3)_2CHCH_2CH_2$–	4.3×10^{-9} M†
12	$CH_3(CH_2)_5$–	3.7×10^{-9} M‡
13	$NH_2(CH_2)_4$–	2.2×10^{-9} M†
14	$NH_2(CH_2)_5$–	5.2×10^{-9} M†
15	 CH_2–	3.9×10^{-8} M†
16	 CH_2CH_2–	3.8×10^{-9} M†
17	 $CH_2CH_2CH_2$–	8.3×10^{-9} M†
18	 CH_2CH_2–	7×10^{-9} M§

Mais ativo que captopril



Ki = 5 x 10^{-10}M

Enalaprilat (MK-422)
I_{50}=1.2 x 10^{-9} M



Enalapril (MK-421)

Table 4 The oral activity deficit of MK–422*

Compound	Enzyme inhibition	Blockade of angiotensin I (in rats) ID_{50} i.v.	Blockade of angiotensin I (± 95% CL) ED_{50} Oral
Captopril	2.0×10^{-8} M	60.5 μg/kg† (51.3–71.6)	0.33 mg/kg
MK-422	1.2×10^{-9} M	8.2 μg/kg (6.6–10.6)	2.29 mg/kg

*Patchett *et al.* (1980).
†More recent results with captopril have given I_{50} values as low as 26.1 g/kg.
CL = confidence limits.

1) Pró-droga: necessidade de hidrólise do éster *in vivo*
2) Absorção via oral: boa
3) Atividade oral: boa
4) Tempo de ação: bom

Merck Sharp & Dohme Research Laboratories

Referências:

Br. J. clin. Pharmac, **18** (1984), 201S–207S

HiperAtivo, 5(1), 6-8, 1998.

Current Opinion in Drug Discovery and Development 1998 **1**(1):4-15

Fundamentos de aplicações de enzimas em transformações químicas

Vá para o vídeo #2