



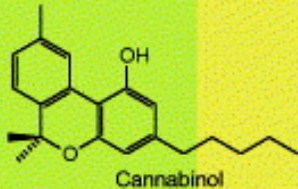
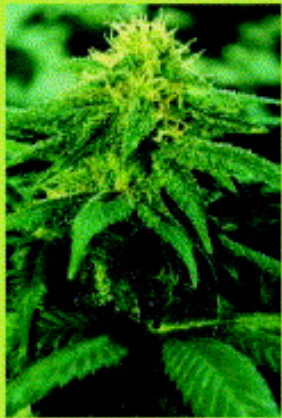
SISTEMA CANABINÓIDE E O CONTROLE DO BALANÇO ENERGÉTICO

TATIANE CRISTINA DE ARAUJO NOGUEIRA

DEPARTAMENTO DE FISILOGIA E BIOFÍSICA
INSTITUTO DE CIÊNCIAS BIOMÉDICAS
UNIVERSIDADE DE SÃO PAULO, SÃO PAULO, BRASIL.

APOIO:  **FAPESP**

SISTEMA CANABINÓIDE



Todd *et al.* (and Adams *et al.* in the USA) fully elucidate and synthesize cannabinol 1940

Wood *et al.* isolate cannabinol from cannabis resin 1899

Cannabinoid pharmacology is thoroughly investigated 1970–1990

Matsuda *et al.* clone the CB₁ receptor 1990

Munro *et al.* clone the CB₂ receptor 1993

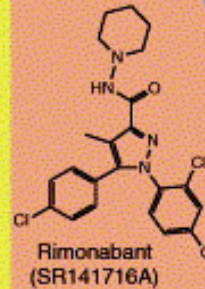
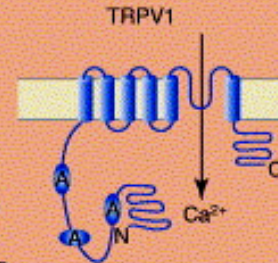
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Sativex® approved for sale in Canada; regulatory approval is filed to sell rimonabant in the USA; the Aberdeen group discovers an allosteric site on CB₁ receptors 2005

New drugs



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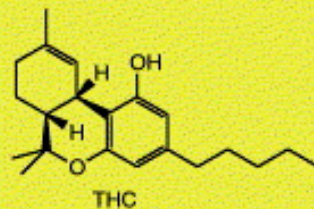
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Cannabis research

1932 Cahn elucidates part of the structure of cannabinol

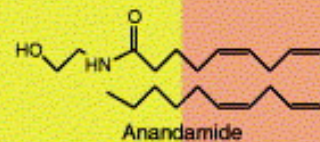
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Cannabinoid research

1988 Howlett's group identifies specific THC binding sites in the brain

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2-AG

Endocannabinoid research

1995 Mechoulam's group and Waku's group identify the second endocannabinoid, 2-AG

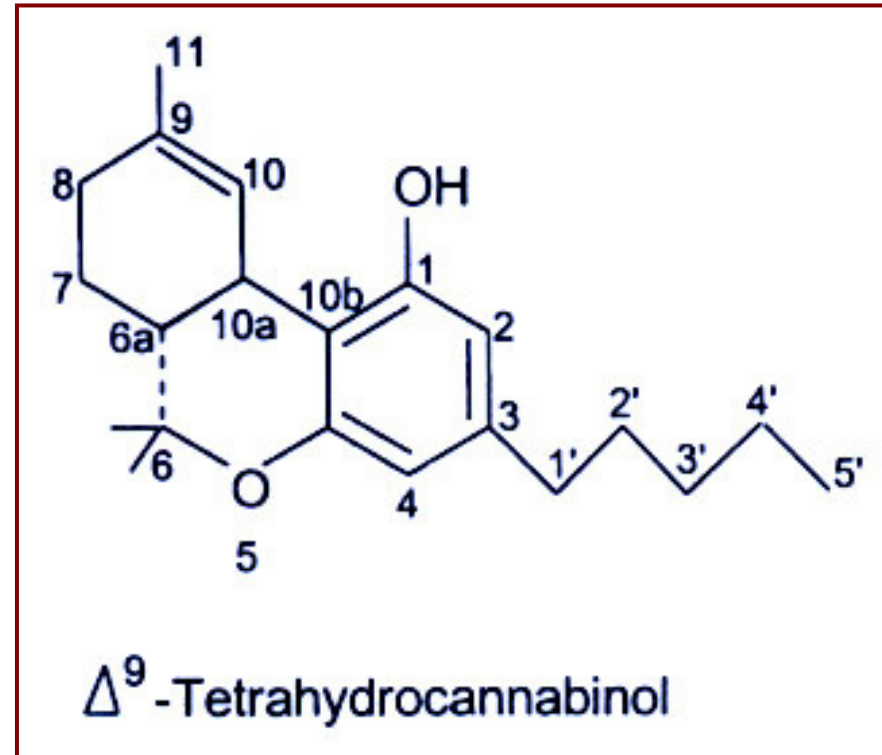
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2003 Bisogno *et al.* clone the first endocannabinoid-biosynthesizing enzymes

???? Cloning of new cannabinoid receptors; identification of other endocannabinoid enzymes; cloning of the endocannabinoid transporter; more endocannabinoid-based therapies



Cannabis sativa



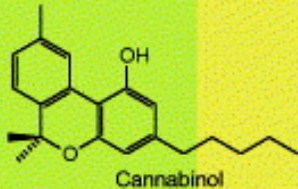
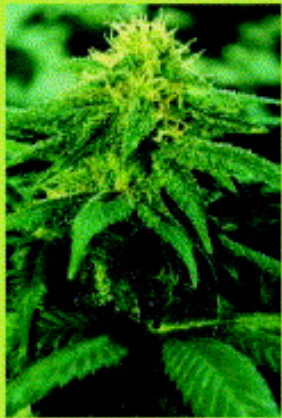
Gaoni & Mechoulam, 1964

CANABINÓIDES

Efeitos Psicoativos X Efeitos Terapêuticos

- ✓ Abuso
- ✓ Tolerância
- ✓ Dependência
- ✓ Toxicidade
- ✓ Prejuízos cognitivos
- ✓ Prejuízos motores

- ✓ Antiemético
- ✓ Analgésico
- ✓ Anticonvulsivante
- ✓ Antipressor intraocular
- ✓ Estimulante do apetite



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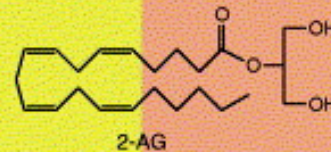
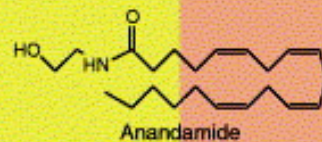
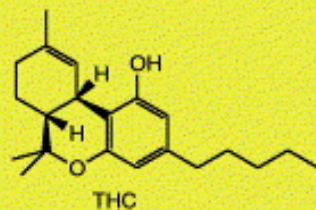
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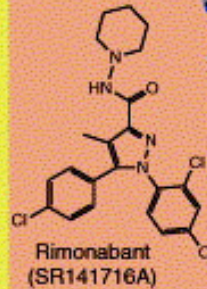
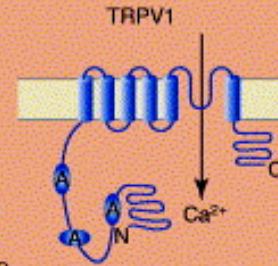


Cannabis research



Cannabinoid research

Endocannabinoid research



New drugs

SISTEMA CANABINÓIDE

Receptores

- **CB1**

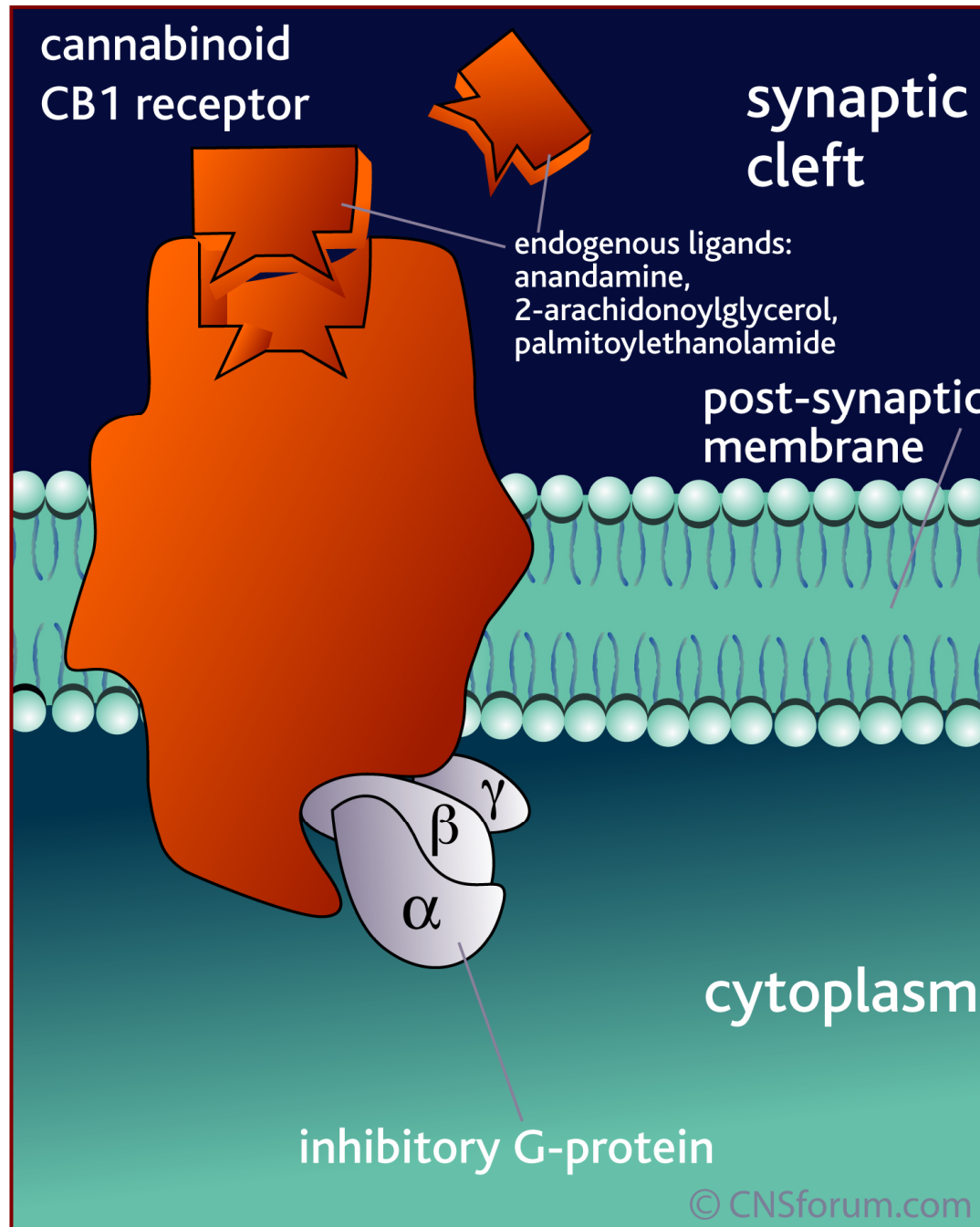
Matsuda *et al.*, 1990

gânglios da base
hipocampo
córtex
cerebelo
núcleo acumbens
tronco encefálico
Hipotálamo
medula espinhal
periferia

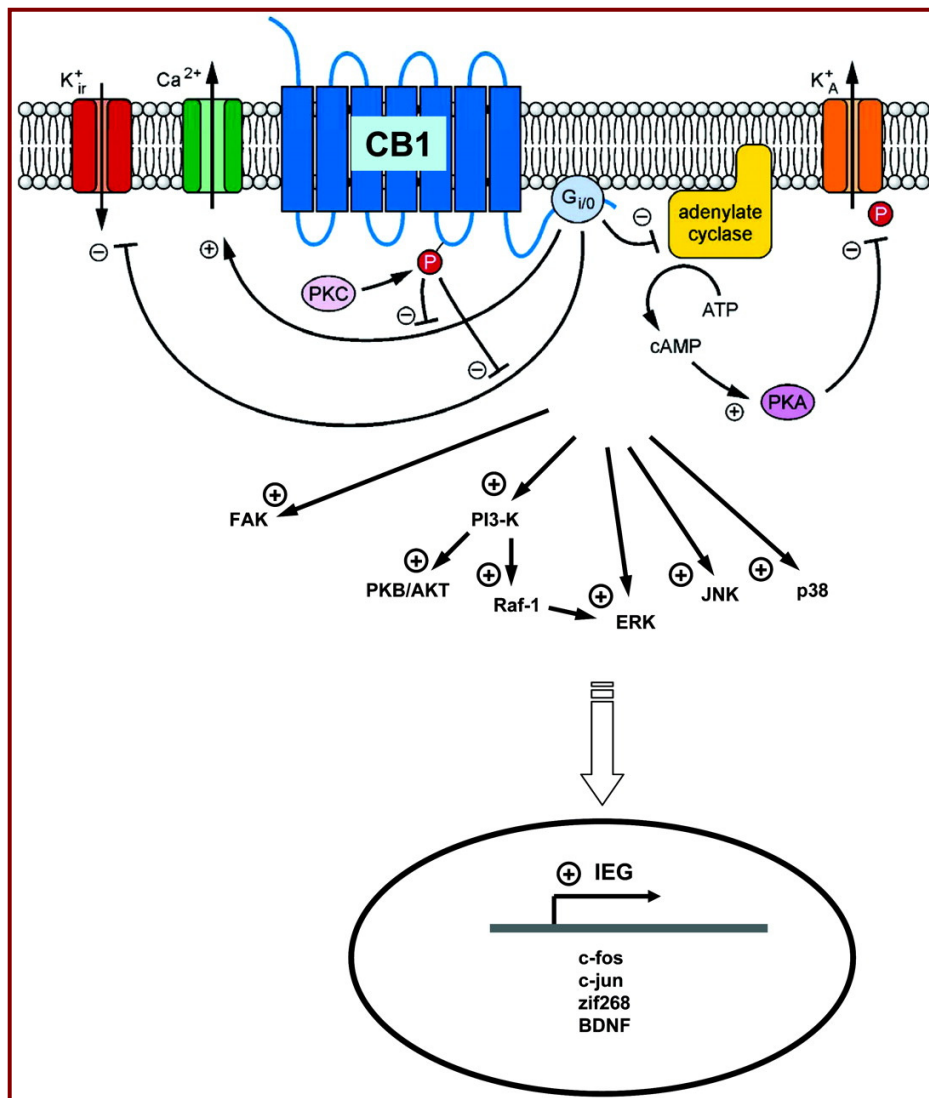
- **CB2**

Munro *et al.*, 1993

baço
tonsilas
sistema imune
SNC



SISTEMA CANABINÓIDE



• CB1

Inibição da adenilato ciclase

Ativação da MAP kinase

Modulação de canais de K⁺ e Ca²⁺

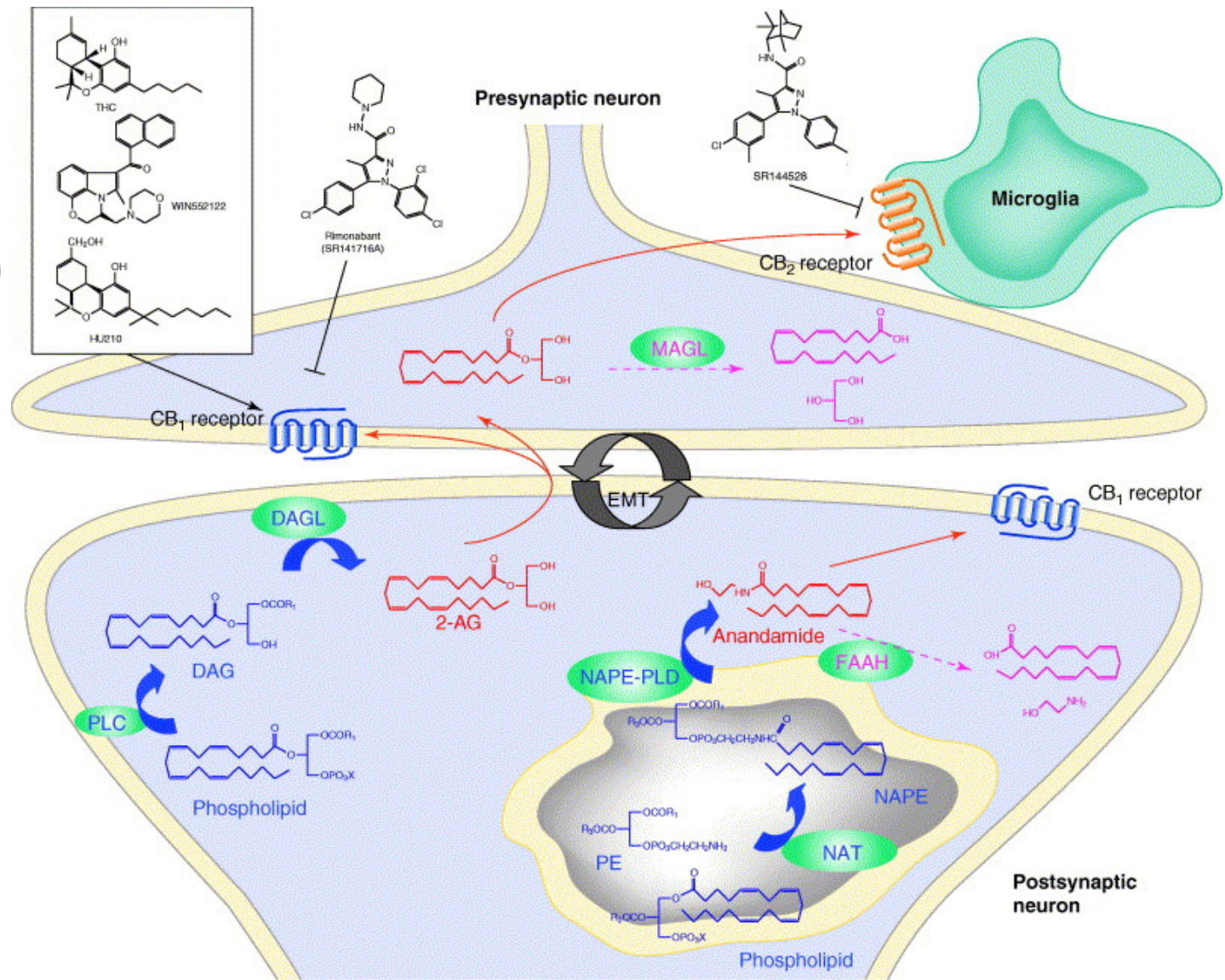
• CB2

Inibição da adenilato ciclase

Ativação da MAP kinase

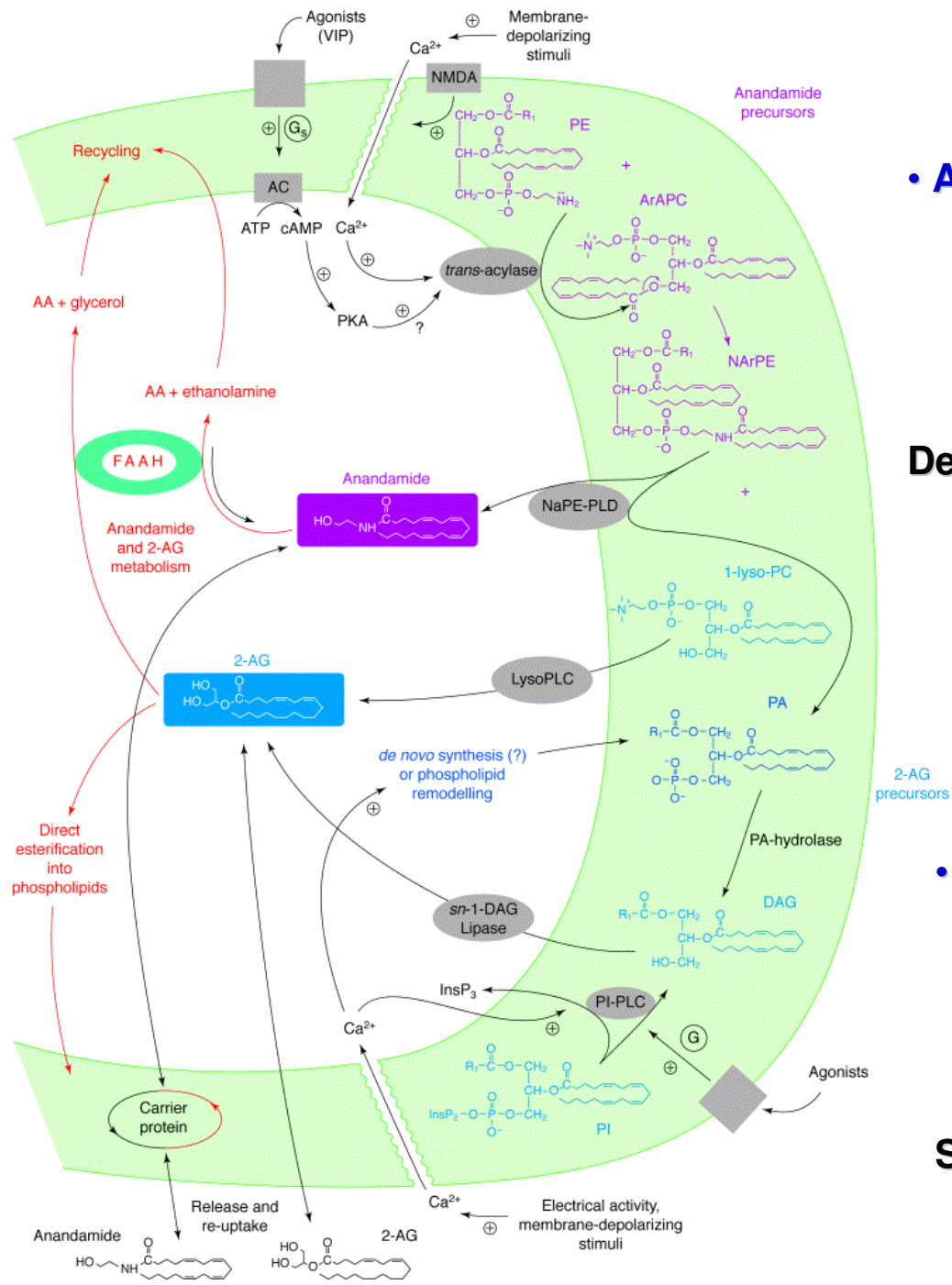
ENDOCANABINÓIDES

Formação e Inativação



AEA – Mechoulam & Pertwee, 1992

2-AG – Mechoulam & Waku, 1995



• **Anandamida (N-araquidoniletanolamina)**

síntese: via NAPE-PLD

degradação: amido hidrolase de ácido graxo (FAAH)

Devane *et al.*, 1992

• **2-AG (2-Araquidilglicerol)**

síntese: via DAG-Lipase

degradação: monoacilglicerol lipase (MAG-Lipase)

Stella *et al.*, 1997

CONTROLE DO BALANÇO ENERGÉTICO

SISTEMA CANABINÓIDE e BALANÇO ENERGÉTICO

CENTRAL X PERIFÉRICO

Δ 9-THC = OREXIGÊNICO

- **Hiperfagia atenuada por SR141716 (ratos)**

Willians *et al.*, 1998

- **Camundongos CB1^{-/-} - Consumo reduzido de alimento após privação.**



Uso do antagonista do CB1 para o tratamento da OBESIDADE.

EFEITO METABÓLICO ?

RECEPTOR CB1

- **Tireóide (embrião e adulto)**

Buckley *et al.*, 1998

- **Adipócitos**

Cota *et al.*, 2003; Bensaid *et al.*, 2003; Engeli *et al.*, 2005

- **Músculo**

Liu *et al.*, 2005

- **Fígado**

Osei-Hyiaman *et al.*, 2005

- **Pâncreas**








Juan-Picó *et al.*, 2006; Bermúdez-Silva *et al.*, 2007; 2008

- **Órgãos reprodutivos**

Pagotto *et al.*, 2006

- **Aferentes vagais (gastrointestinal)**

Burdyga *et al.*, 2003

	Site of action	Effect of CB ₁ activation	Effects of a HFD	Potential consequences
	Epididymal adipose tissue	<ul style="list-style-type: none"> ↑ Lipogenesis (LDL, FAS) ↑ PPAR-γ expression ↑ Glucose uptake ↓ AMPK activity ↓ Adiponectin 	<ul style="list-style-type: none"> ↑ 2-AG Sustained 	<ul style="list-style-type: none"> ↑ Energy storage ↑ Dyslipidaemia ↑ Insulin resistance ↑ Excessive visceral fat
	Subcutaneous adipose tissue	See above	<ul style="list-style-type: none"> ↓ 2-AG, AEA Sustained 	<ul style="list-style-type: none"> ↓ Subcutaneous fat ↑ Visceral and ectopic fat
	Pancreas	<ul style="list-style-type: none"> ↑ Insulin release? 	<ul style="list-style-type: none"> ↑ 2-AG, AEA Early and sustained 	<ul style="list-style-type: none"> ↑ Hyperinsulinaemia?
	Liver	<ul style="list-style-type: none"> ↑ Fatty acid synthesis ↓ AMPK activity 	<ul style="list-style-type: none"> ↑ AEA ↑ CB₁ Early 	<ul style="list-style-type: none"> ↑ Dyslipidaemia ↑ Dyslipoproteinaemia ↑ Steatosis
	Skeletal muscle	<ul style="list-style-type: none"> ↓ AMPK activity? ↓ Glucose uptake? ↓ Glucose oxidation? 	<ul style="list-style-type: none"> ↑ 2-AG Early ↑ CB₁ 	<ul style="list-style-type: none"> ↑ Insulin resistance? ↓ Energy expenditure
	Heart		<ul style="list-style-type: none"> ↑ 2-AG, AEA Early and sustained 	<ul style="list-style-type: none"> ↑ Pericardial fat? ↑ Cardiovascular risk?
	Kidneys	<ul style="list-style-type: none"> ↓ Glomerular filtration 	<ul style="list-style-type: none"> ↑ 2-AG, AEA Late and sustained 	<ul style="list-style-type: none"> ↑ Hypertrophy? ↑ Perirenal fat? ↑ Renal failure



SR141716A

Rimonabant

(Acomplia, Zimulti, Slimona)

Sanofi-Aventis

Taranabant

Merck

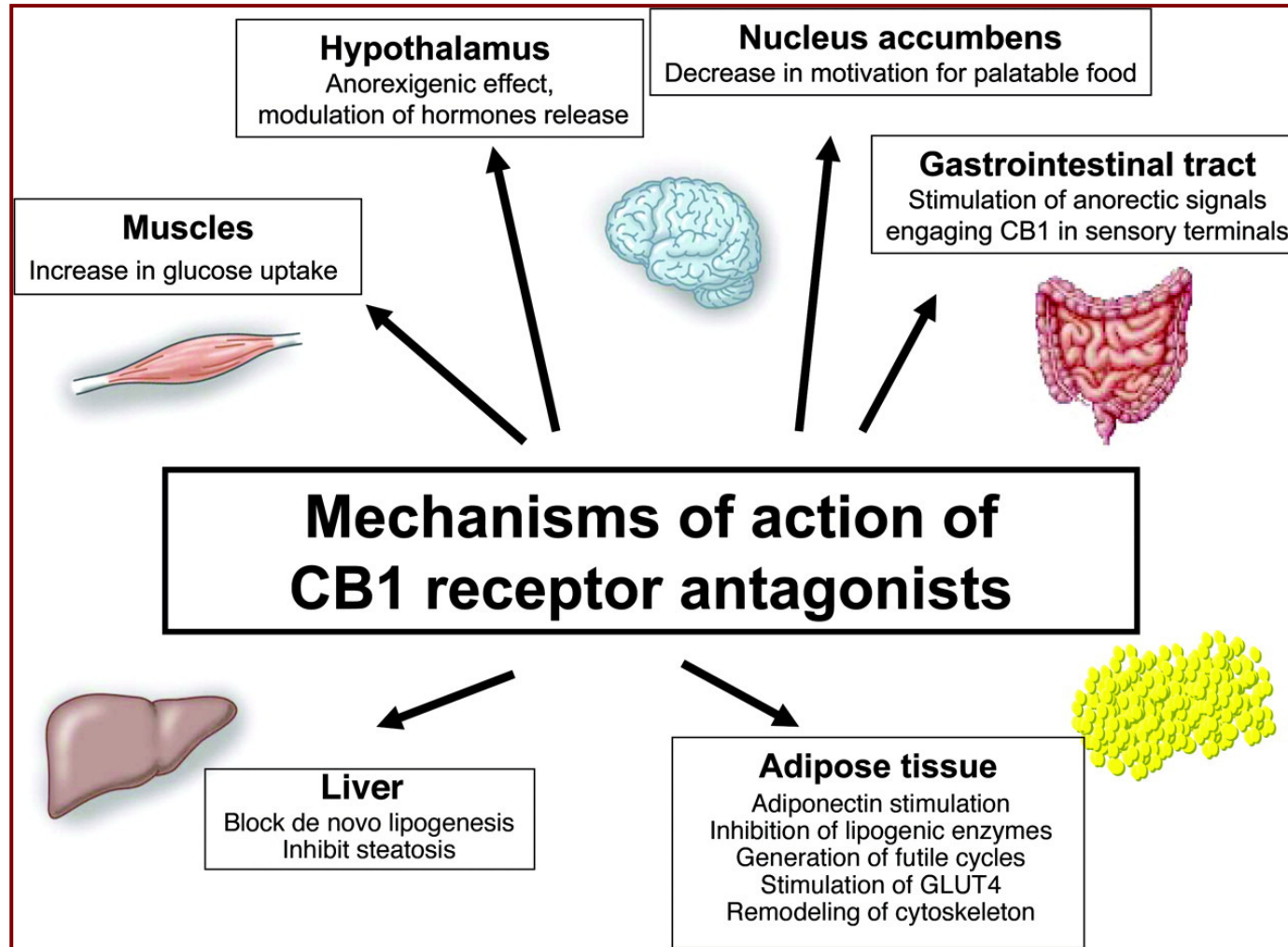
Aprovado pela Anvisa (Agência Nacional de Vigilância Sanitária)

Abril de 2007.

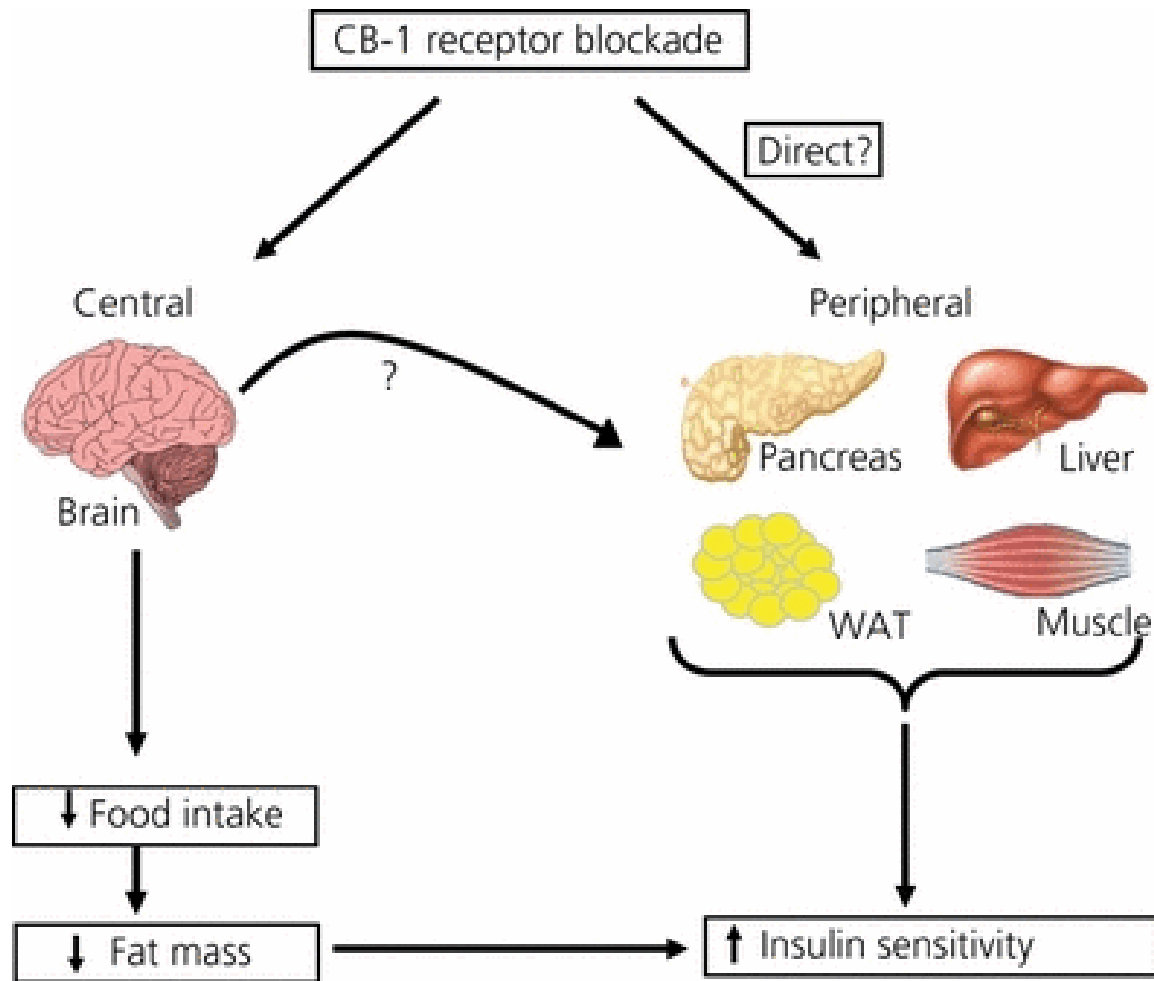
Liberado pela Cmed (Câmara de Regulação do Mercado de Medicamentos)

Julho de 2007.

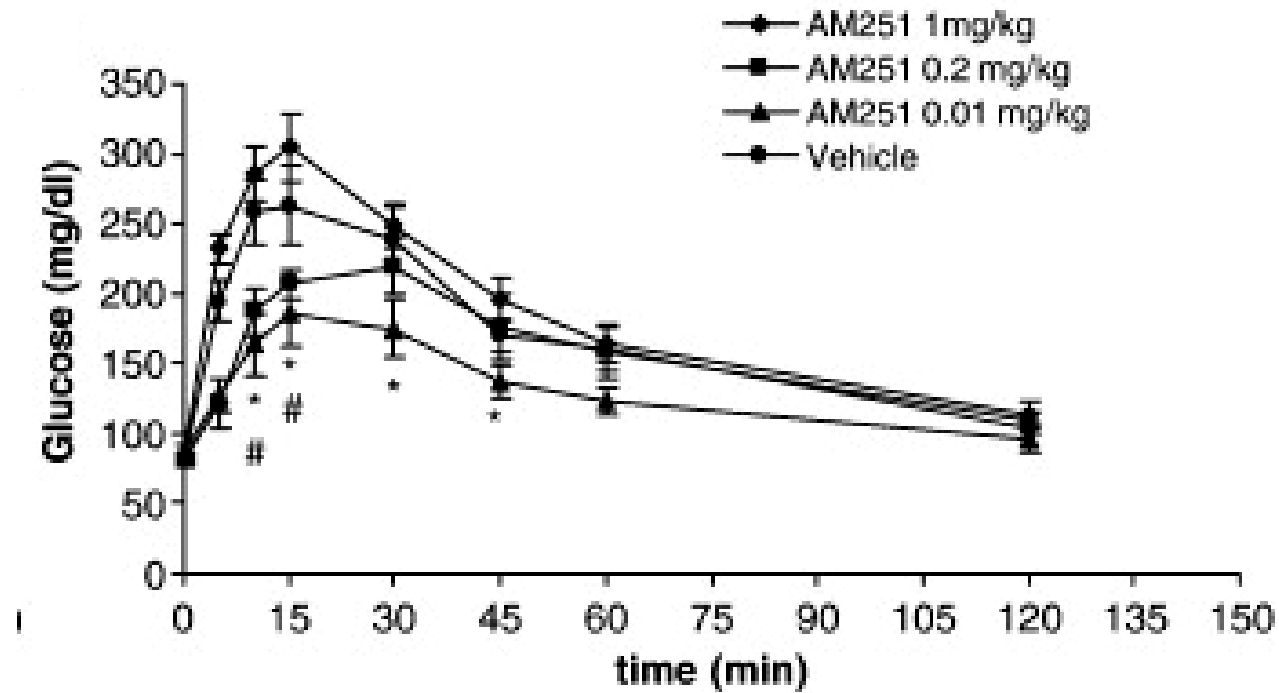
Efeitos do antagonista do CB1 em órgãos envolvidos no controle do metabolismo



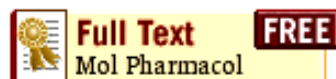
Pagotto *et al.*, 2006



Nogueiras *et al.*, 2008



Bermudez-Silva FJ *et al.*, 2007



The cannabinoid CB1 receptor antagonist Rimonabant stimulates 2-deoxyglucose uptake in skeletal muscle cells by regulating phosphatidylinositol-3-kinase activity.

[Esposito I](#), [Proto MC](#), [Gazzerro P](#), [Laezza C](#), [Miele C](#), [Alberobello AT](#), [D'Esposito V](#), [Beguinot F](#), [Formisano P](#), [Bifulco M](#)

DBPCM & IEOS, CNR, Universita di Napoli "Federico II"

The endocannabinoid system regulates food intake, energy and glucose metabolism at both central and peripheral level. We have investigated the mechanism by which it may control glucose uptake in skeletal muscle cells. Detectable levels of the cannabinoid receptor type 1 (CB1) were revealed in L6 cells. Exposure of differentiated L6 myotubes to the CB1 antagonist Rimonabant (SR141716) selectively increased 2-deoxyglucose uptake (2-DG) in a time- and dose-dependent manner. A similar effect was induced by genetic silencing of CB1 by siRNA. Protein expression profiling revealed that both the regulatory p85 and the catalytic p110 subunits of the phosphatidylinositol-3-kinase (PI3K) were increased by SR141716. No significant change in the cellular content of other known molecules regulating PI3K was observed. However, PDK-1, Akt/PKB and PKCzeta activities were rapidly induced following SR141716 treatment of L6 cells in a PI3K-dependent manner. The stimulatory effect of SR141716 on PI3K expression and activity was largely prevented by H-89, an inhibitor of the cAMP-dependent protein-kinase. Moreover, SR141716-stimulated 2-DG uptake was blunted by the co-incubation either with H-89 or with the PI3K inhibitor LY294002, both in L6 cells and in mouse primary myocytes. Thus, modulation of CB1 regulates glucose uptake at the level of the PI3K signalling system in skeletal muscle cells. Interfering with CB1 signalling may therefore ameliorate gluco-regulatory functions in peripheral tissues.

PMID: 18801918 [PubMed - as supplied by publisher]



EFEITOS DA ADMINISTRAÇÃO AGUDA DO ANTAGONISTA CANABINÓIDE AM251 SOBRE A SINALIZAÇÃO DA INSULINA.

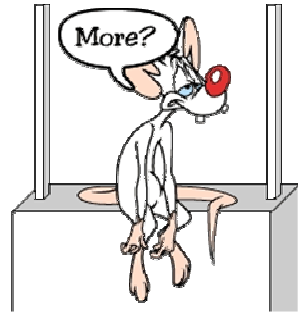
NOGUEIRA, TC; ANHÊ GF; TORRÃO AS; BORDIN S.

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APOIO:  **FAPESP**

ESTUDO *IN VIVO*

Jejum de 16h



Injeção intraperitoneal do veículo (DMSO em salina), do AM251 ou AM630 (0,2 e 0,05 mg/Kg, respectivamente) 30 min antes dos testes.



GTT (2g/Kg peso)

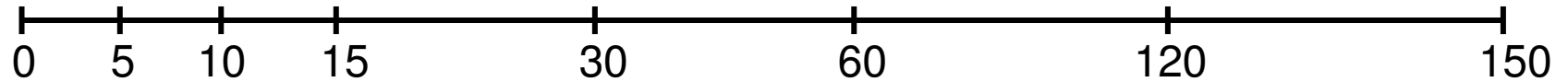


ITT (25mU/Kg peso)

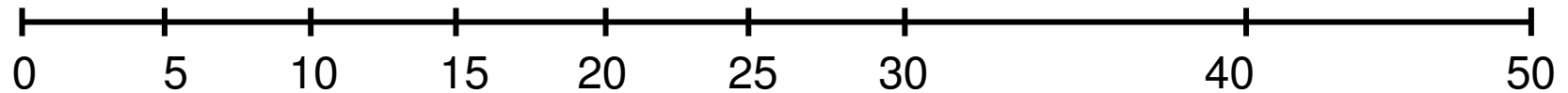


BLOTTING

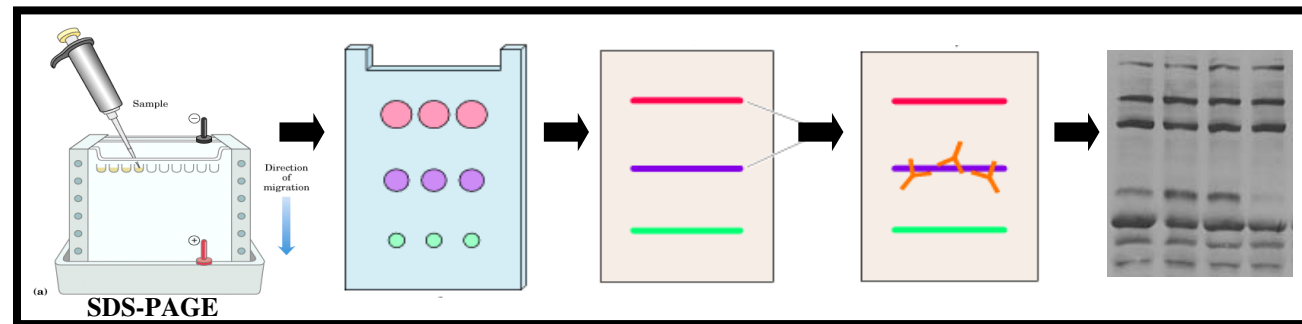
GTT



ITT

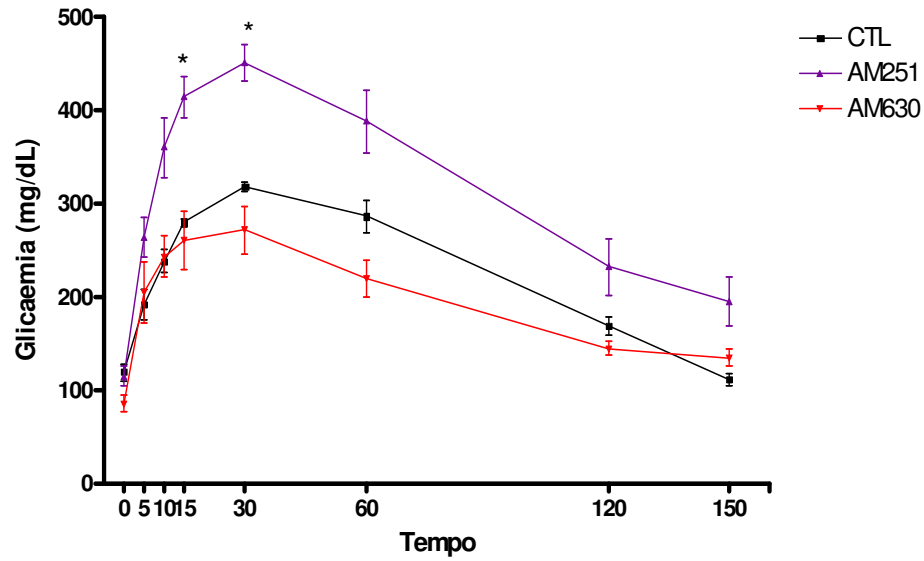


**FÍGADO
MÚSCULO
TEC. ADIPOSO**
Antes e após a
injeção de insulina

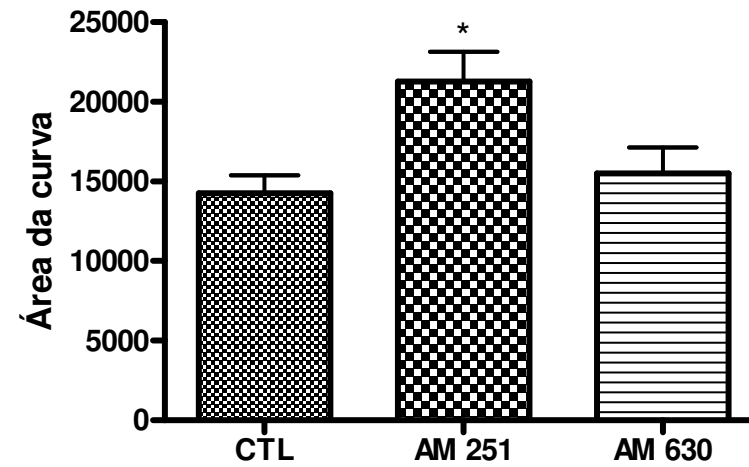


Fosforilação do IR – IRS e AKT (Ser 473)

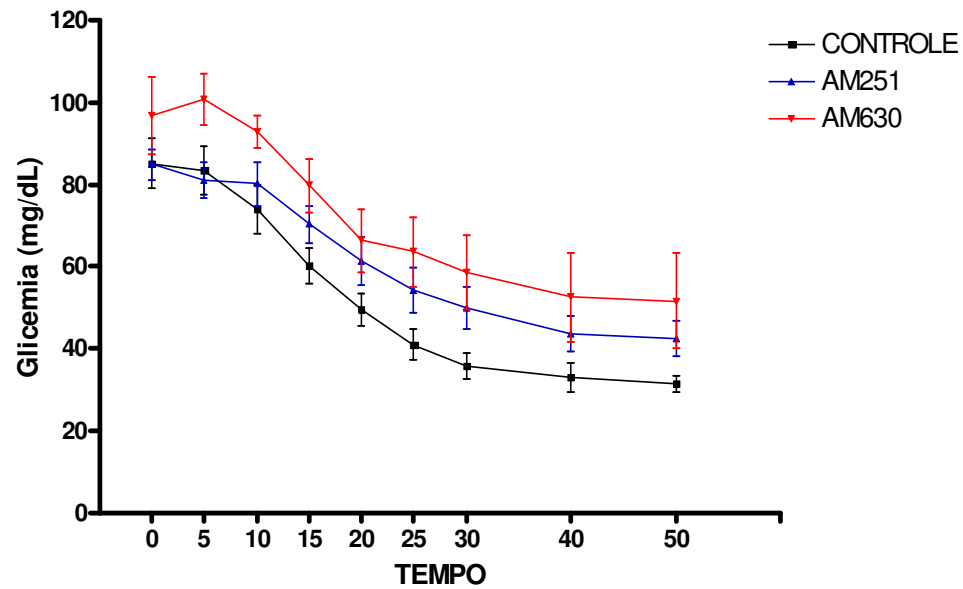
GTT



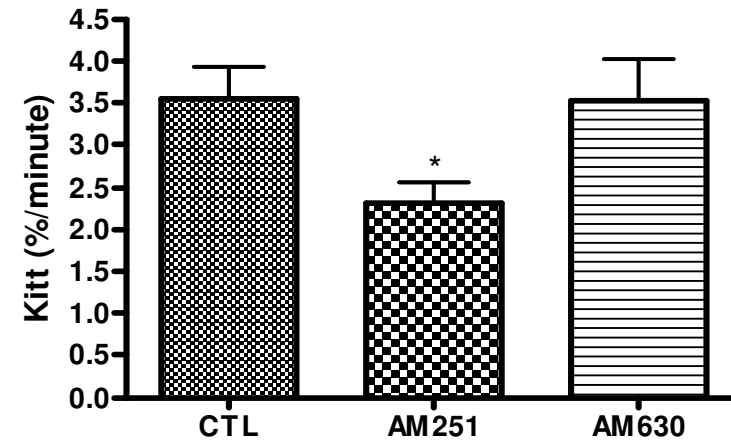
Area da curva

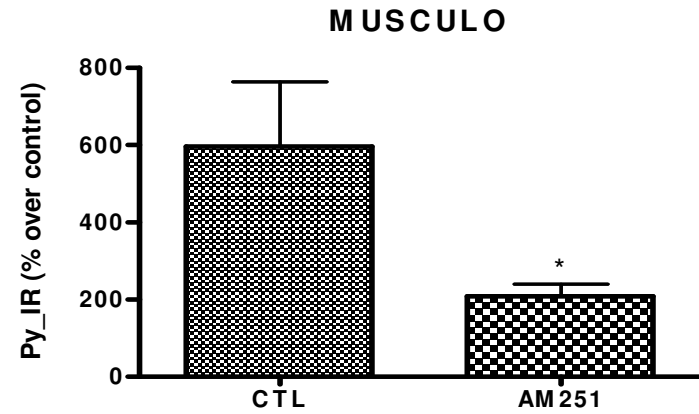
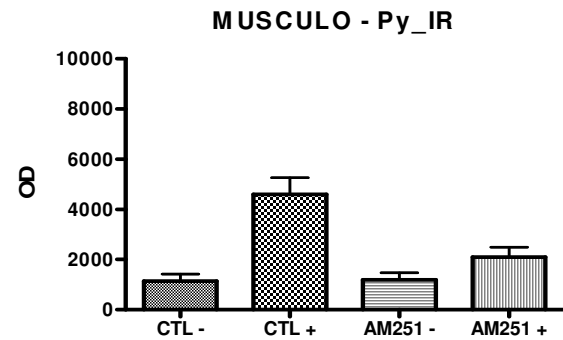
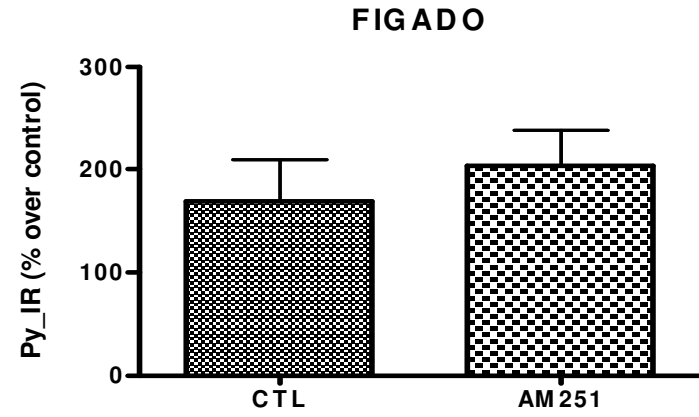
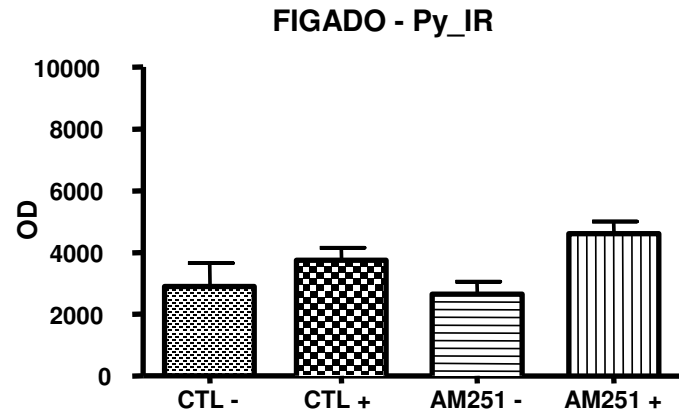
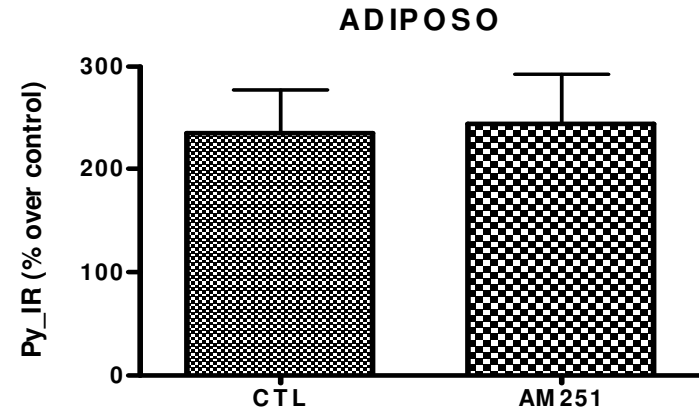
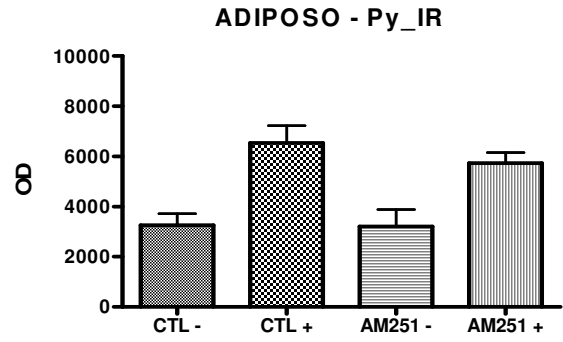


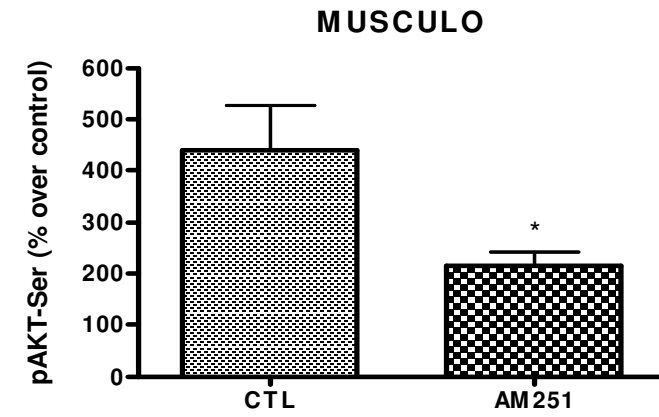
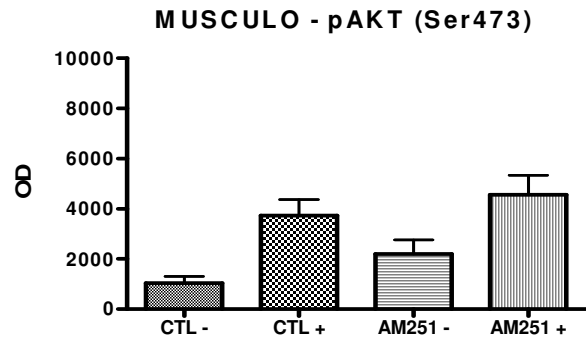
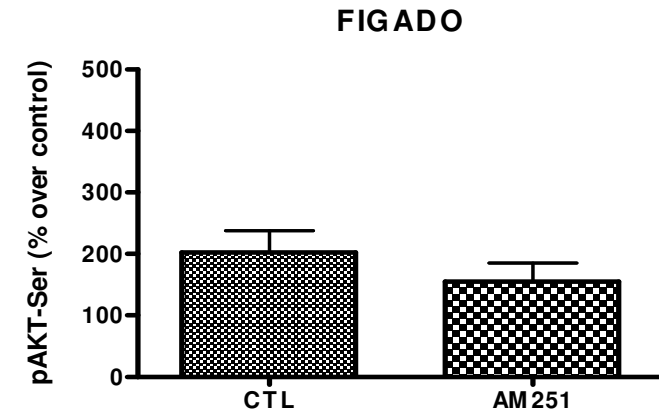
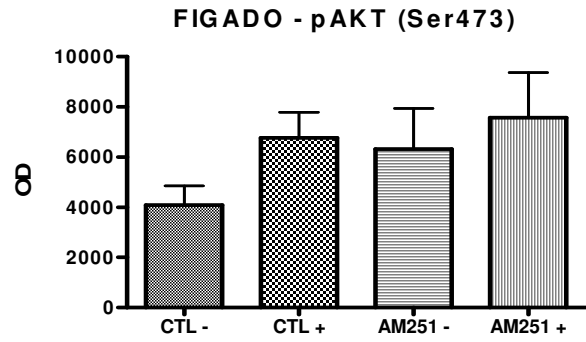
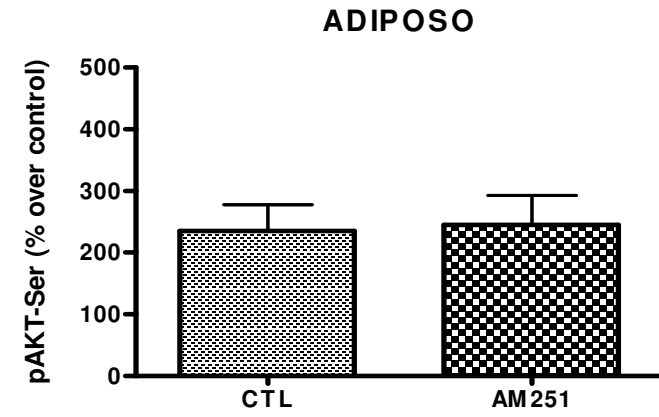
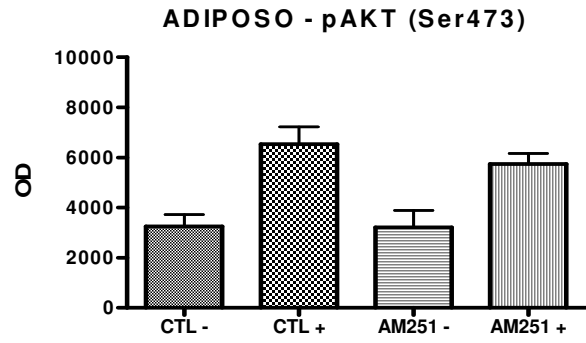
ITT



Kitt



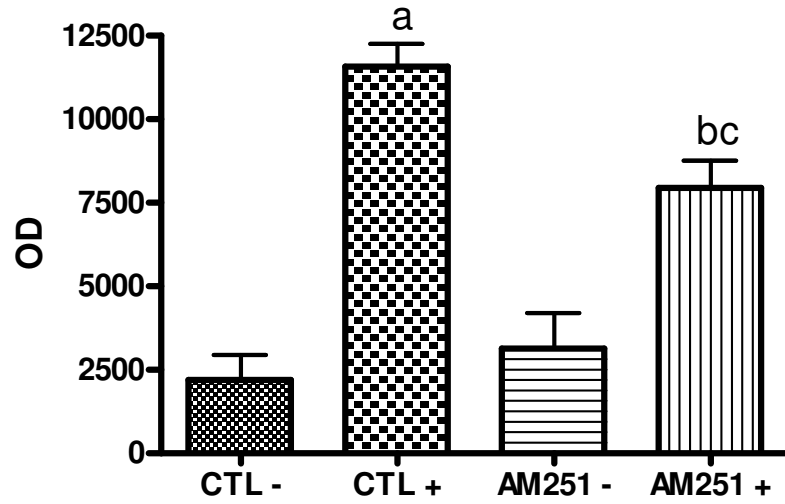




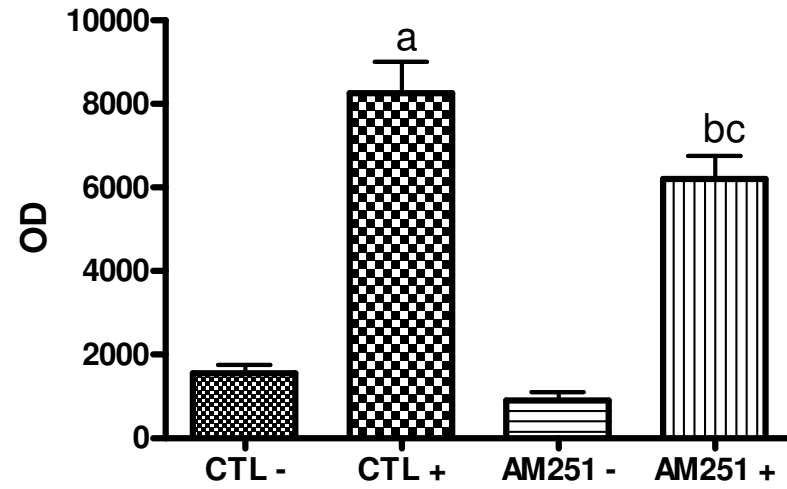
ESTUDO *IN VITRO*

CULTIVO de L6

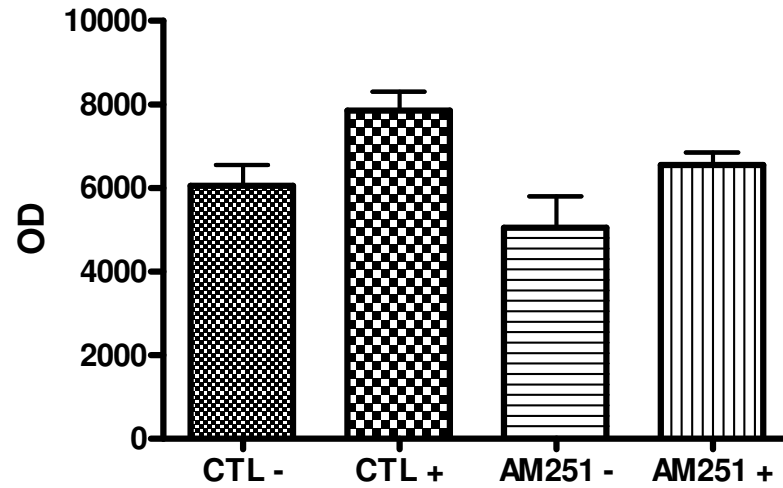
pAKT(Ser473)



Py_IRS



Py_IR

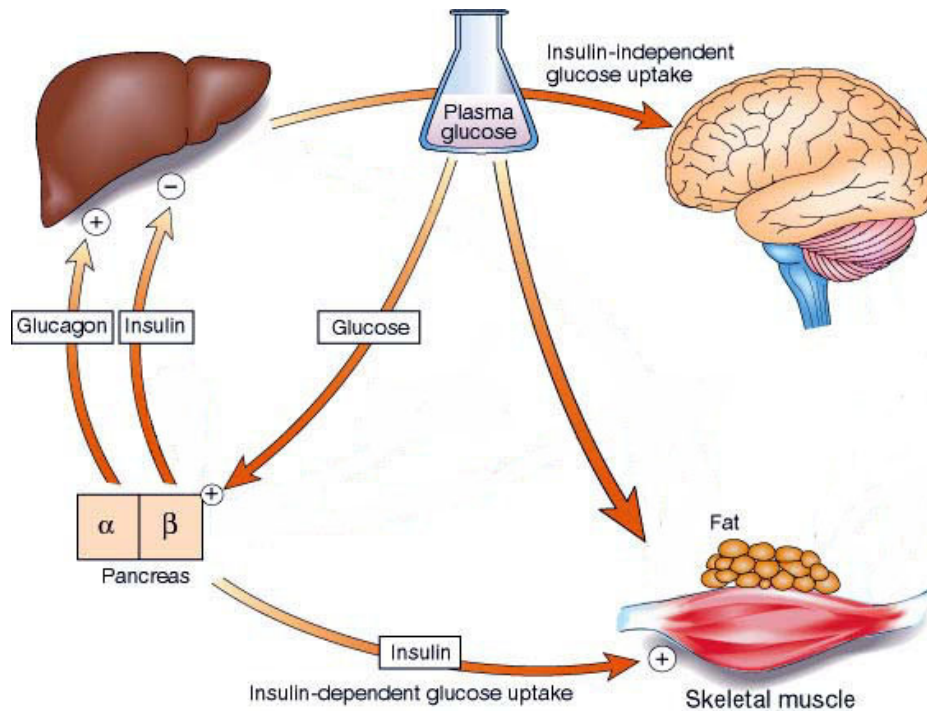


CONCLUSÕES

1. O inibidor do receptor CB1 AM251 induziu intolerância à glicose *in vivo* e diminuiu a resposta periférica à insulina agudamente.
2. Este efeito parece ser resultado da diminuição da sinalização da insulina (pAKT, Py_IR e Py_IRS) em músculo (*in vivo* e *in vitro*).
3. Além disso, este parece ser um efeito dose-dependente e possivelmente espécie-dependente.

OBESIDADE & DIABETES

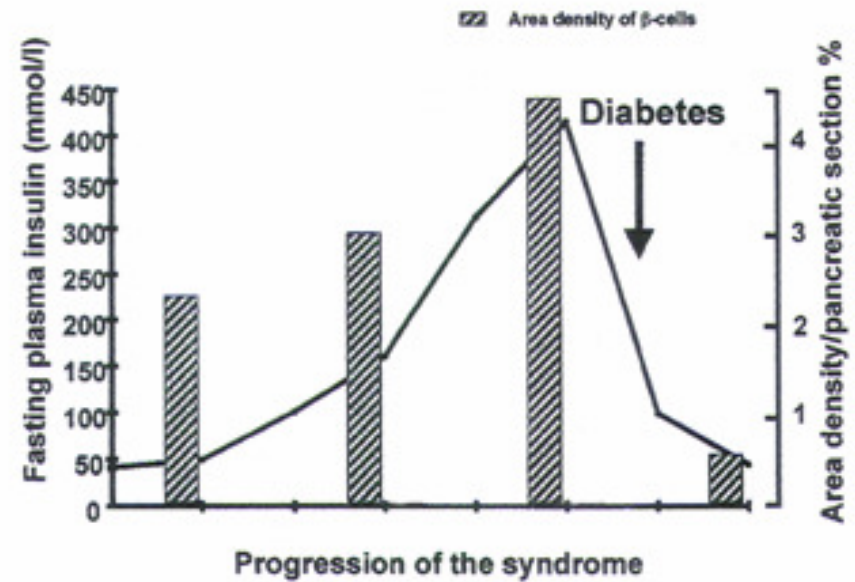
HOMEOSTASIA GLICÊMICA



Resistência à insulina



Aumento da secreção



RETÍCULO ENDOPLASMÁTICO (RE): Síntese, dobramento, montagem, formação de pontes de dissulfeto e glicosilação de proteínas.

O RE é altamente sensível à **alterações** que perturbem os níveis energéticos celulares, o estado redox e as concentrações de cálcio.



↓ CAPACIDADE DE PROCESSAMENTO DE PROTEÍNAS



ACUMULO E AGREGAÇÃO de *UNFOLDED PROTEINS*
Tóxico para célula (isquemia, doenças neurodegenerativas e diabetes)



ESTRESSE DE RETÍCULO



ESTRATÉGIAS DE PROTEÇÃO PARA COMBATER OS EFEITOS DELETÉRIOS



UNFOLDED PROTEIN RESPONSE (UPR)

UNFOLDED PROTEIN RESPONSE (UPR)

RESPOSTA ADAPTATIVA IMEDIATA:

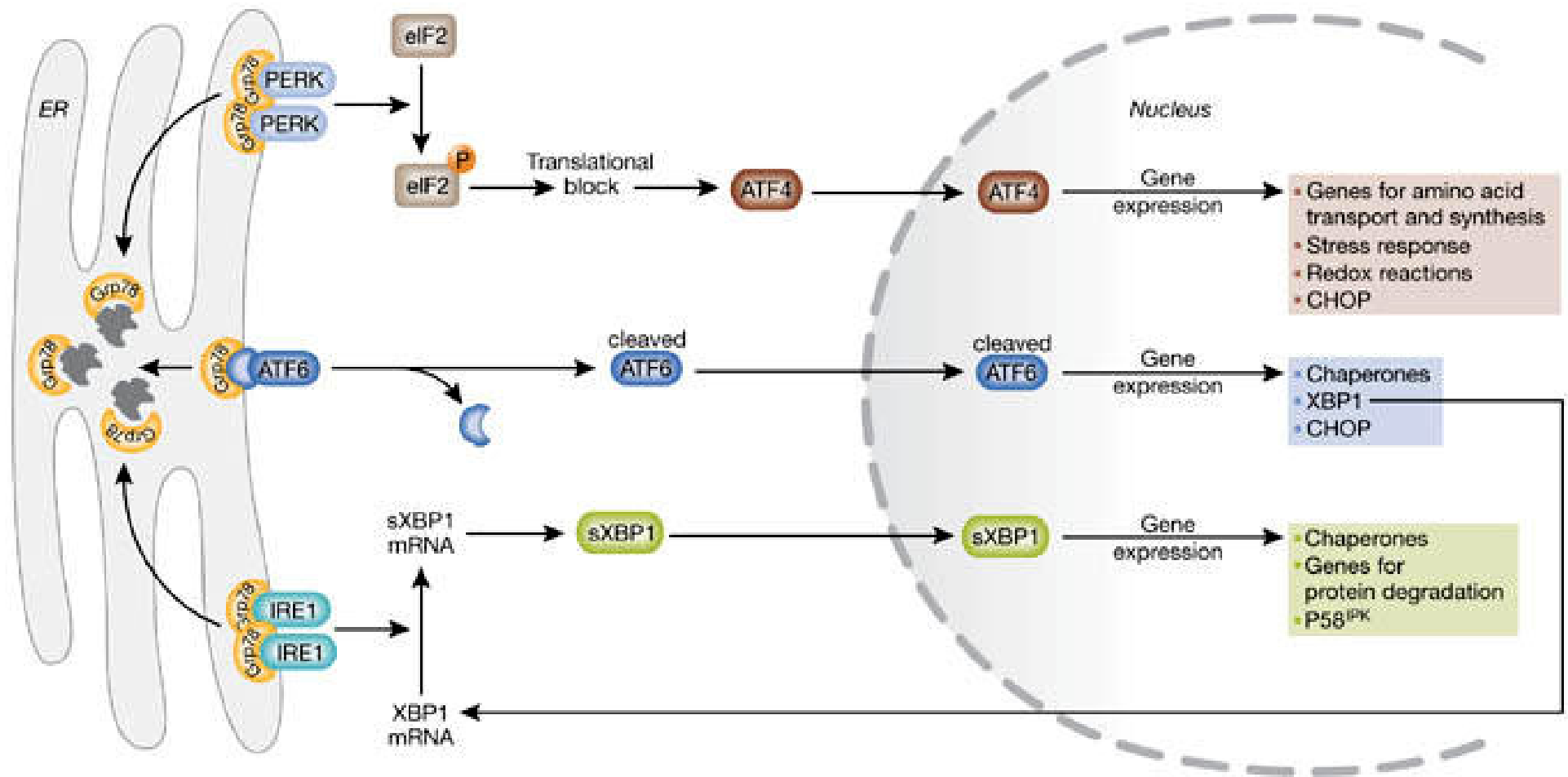
- Atenuação da taxa de tradução
- Aumento da expressão de chaperonas e foldases residentes no RE
- Ativação de um sistema de degradação associado ao RE (ERAD)

ALARME:

- Ativação de NFkB: induz a expressão de genes mediadores da resposta de defesa

SUICÍDIO CELULAR:

- Tipicamente **APOPTOSE** a fim de descartar as células com disfunção irreversível



**THERE ARE PHYSIOLOGIC
PERTUBATIONS OF HOMEOSTASIS THAT
CAN TRIGGER ENDOCANNABINOIDS
PRODUCTION, WHICH, TOGETHER WITH
OTHER HOMEOSTATIC SIGNALLING
SYSTEMS, HELPS CELLS RETURN TO
THEIR STEADY STATE.**

Di Marzo V (2008) Diabetologia.

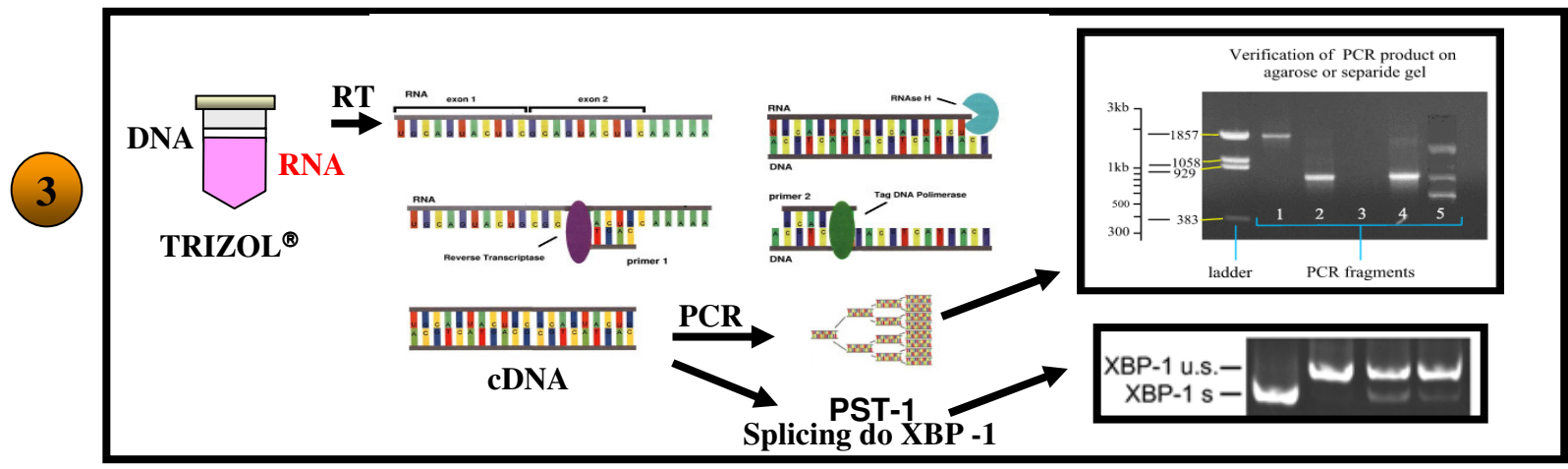
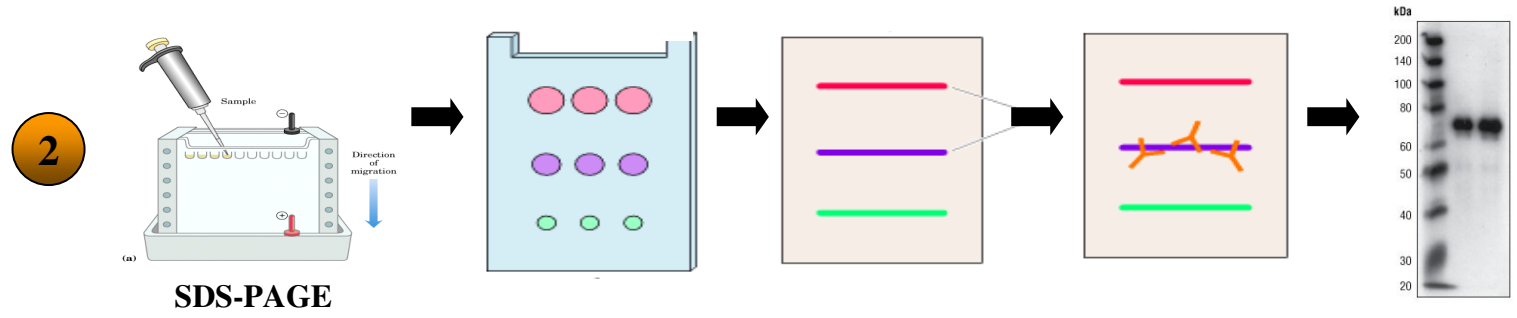
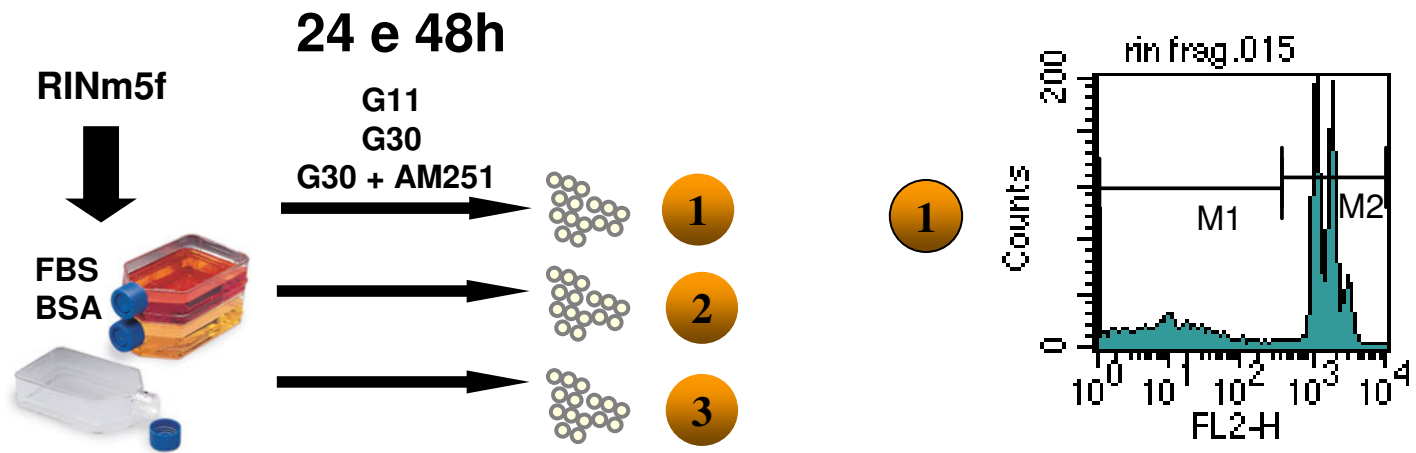


O ANTAGONISTA CANABINÓIDE AM251 PROTEGE A CÉLULA BETA PANCREÁTICA DO ESTRESSE DE RE INDUZIDO POR GLICOSE.

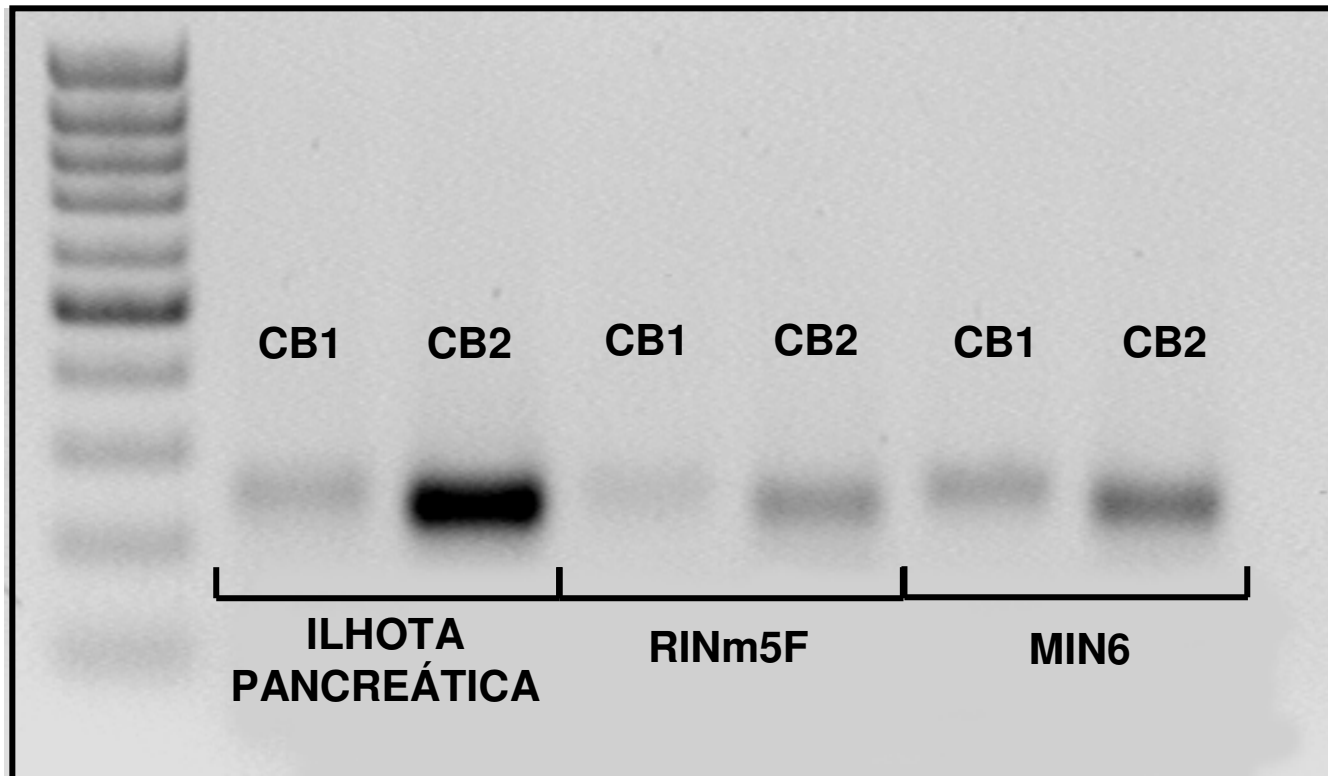
NOGUEIRA, TC; ANHÊ GF; LELLIS-SANTOS C; TORRÃO AS;
CIPOLLA-NETO J; BORDIN S.

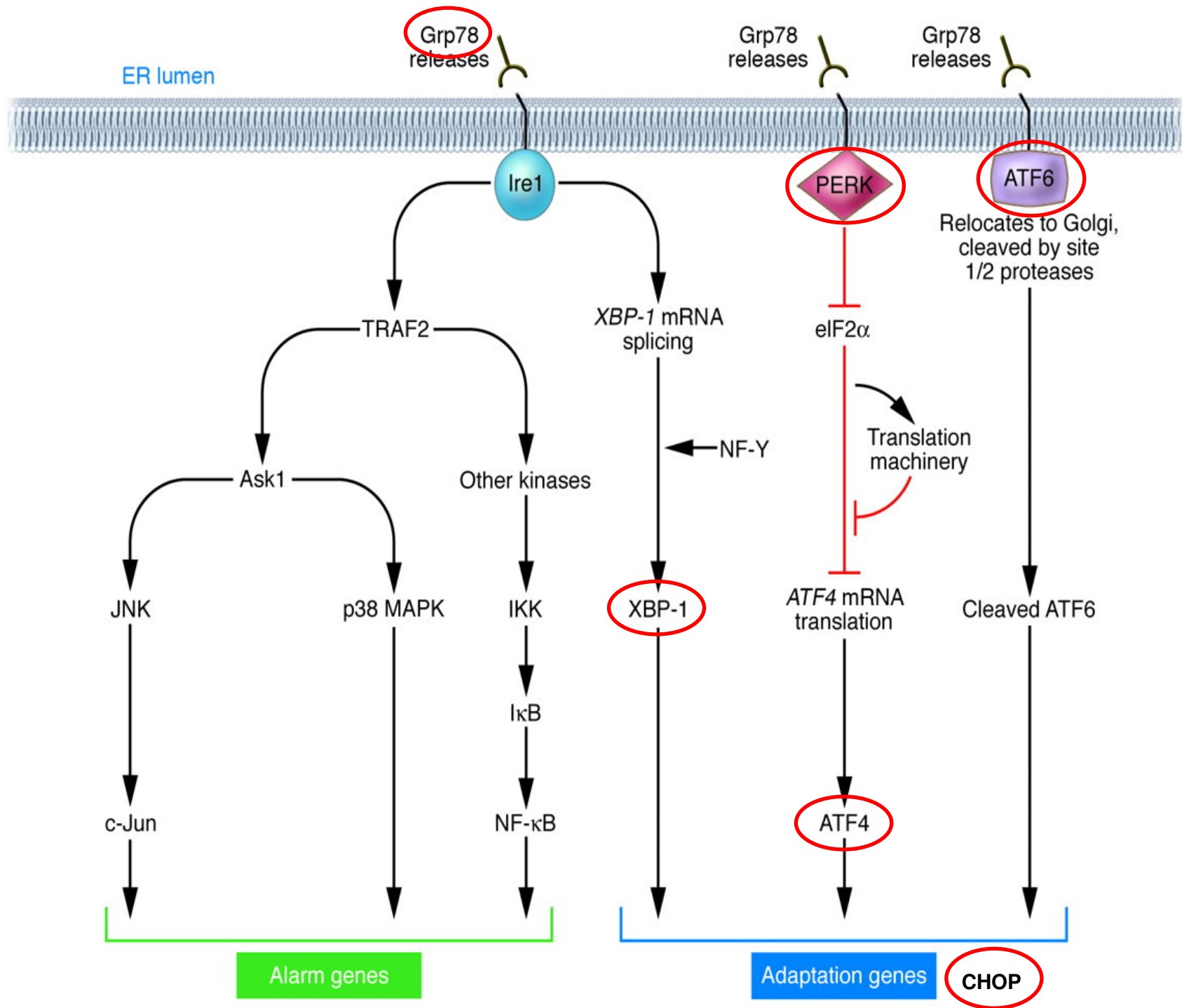
DEPARTAMENTO DE FISIOLOGIA E BIOFÍSICA
INSTITUTO DE CIÊNCIAS BIOMÉDICAS
UNIVERSIDADE DE SÃO PAULO, SÃO PAULO, BRASIL.

APOIO:  FAPESP



mRNAs de receptores CB1 e CB2 são expressos em Ilhotas Pancreáticas e células RINm5F e MIN6.





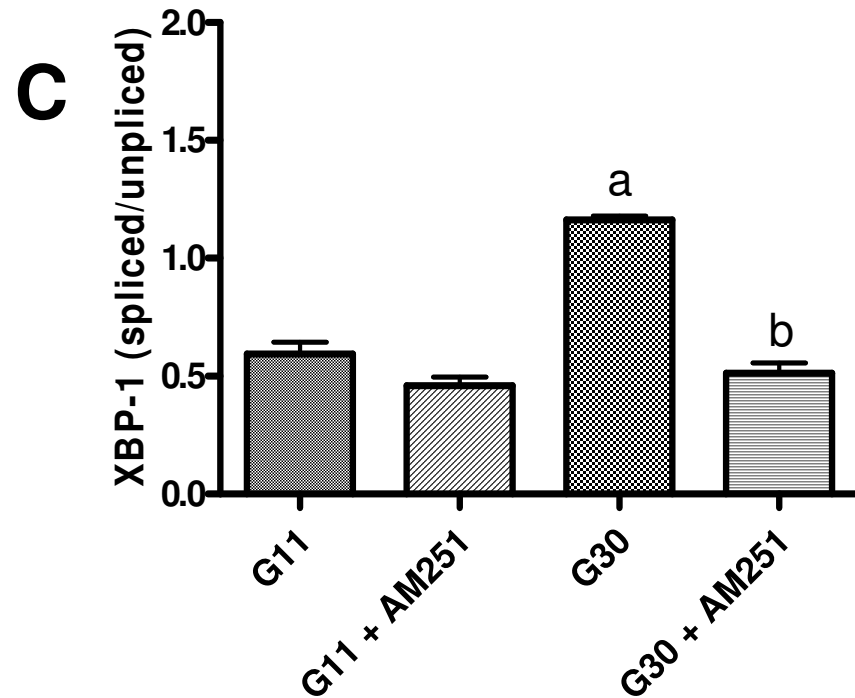
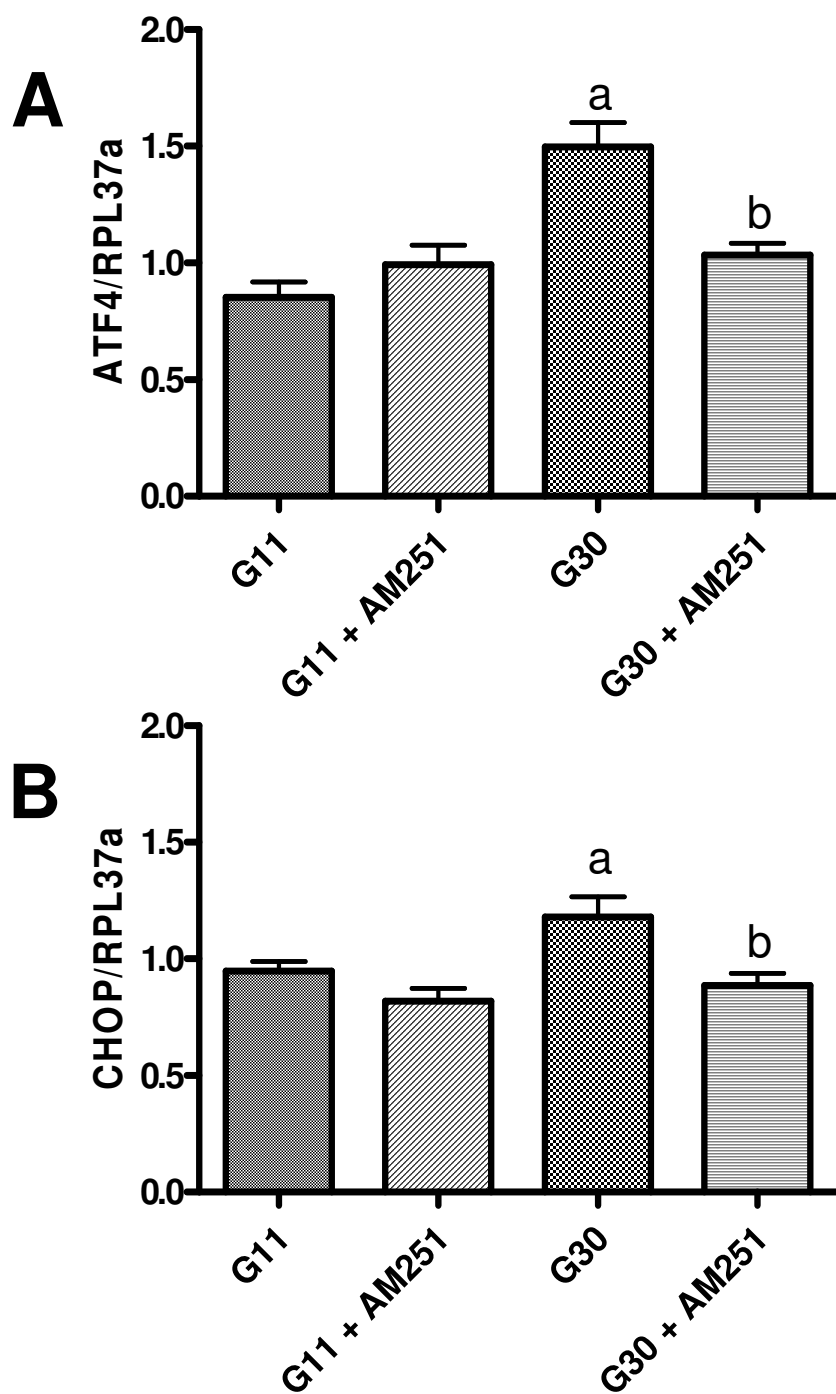


Figura 2 – Cultura de célula RINm5F em meio com BSA 0,1%. G30 induziu um aumento na expressão de ATF4 (**A**) e CHOP (**B**) (respectivamente $20 \pm 0.8\%$, $62 \pm 1,3\%$) e *splicing* do XBP-1 (**C**) ($93 \pm 1\%$). O tratamento combinado das células com G30 e AM251 preveniu estas alterações. a: $P < 0.001$ vs G11; b: $P < 0.001$ vs G30

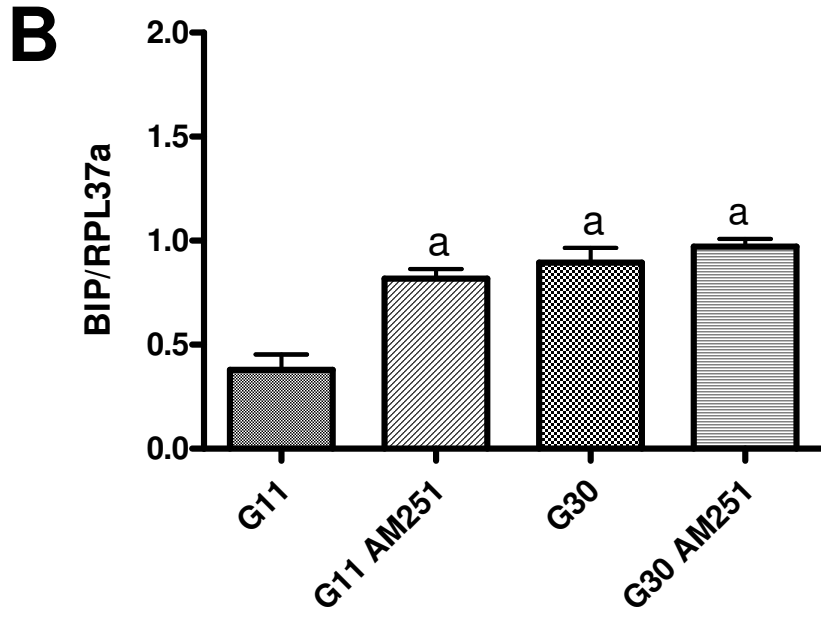
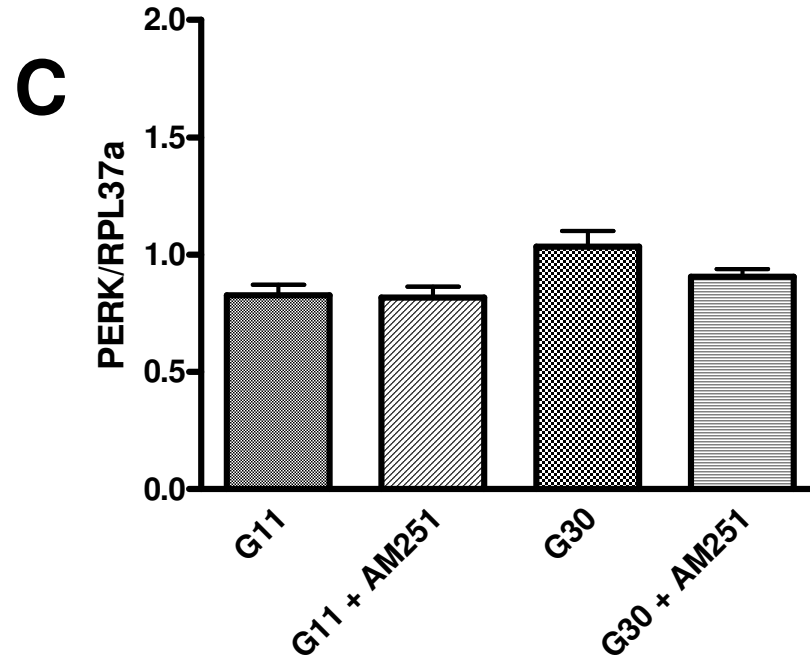
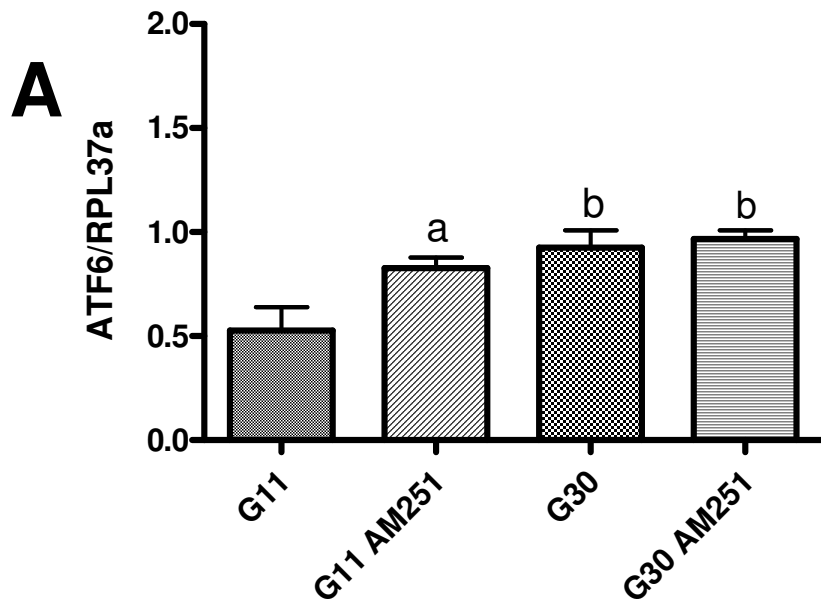


Figura 3 – A expressão de ATF6 (A) e BIP (B) foi aumentada na presença de G30 ($85\pm 0.9\%$ e $112\pm 0.9\%$, respectivamente) e AM251 ($76\pm 0,6\%$ e $91\pm 0.7\%$, respectivamente) mas não houve aumento adicional na presença de ambos G30 e AM251. A expressão da PERK não foi alterada por qualquer tratamento.
 (A) a: $P < 0.001$ vs G11.
 (B) a: $P < 0.05$ vs G11; b: $P < 0.01$ vs G11.

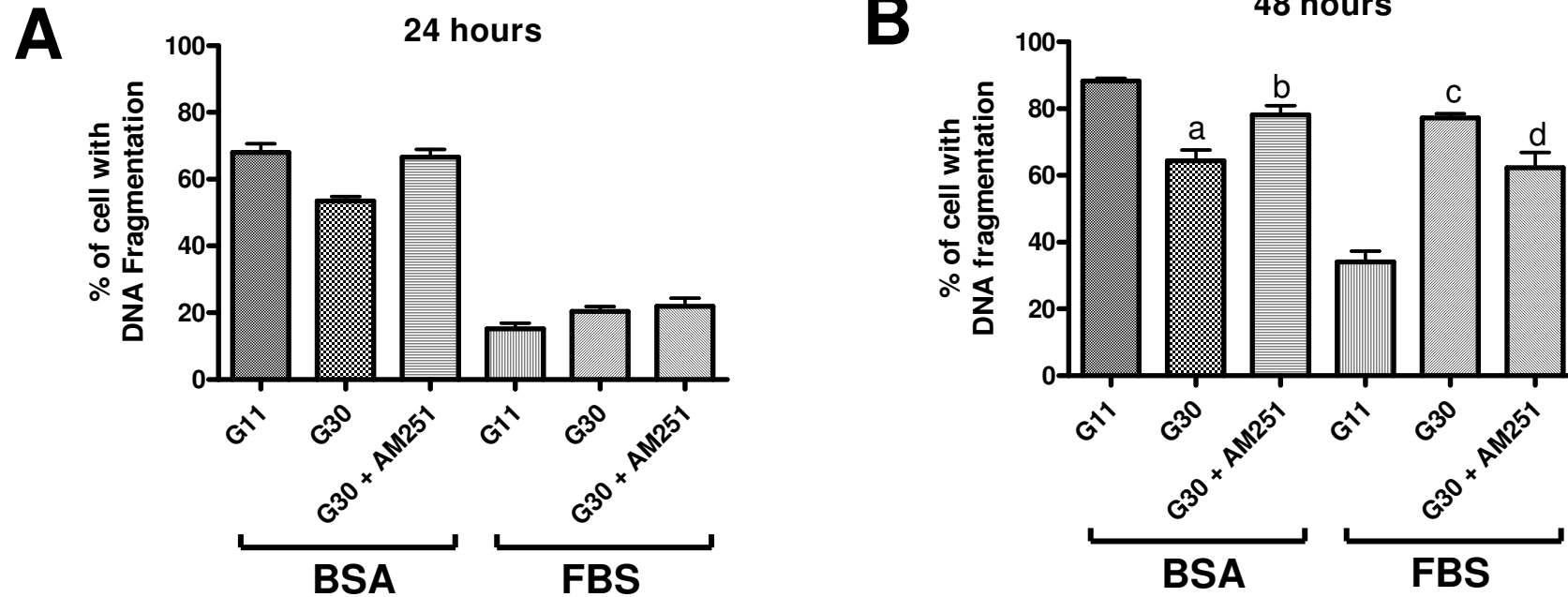


Figura 4 – (A) Glicose 30 mM (G30) ou G30 e AM251 não modularam a fragmentação de DNA em células RINm5F cultivadas durante 24h em meio contendo FBS 10% ou em meio contendo BSA 0,1% (B) Quando as células foram cultivadas durante 48h, G30 diminuiu a % de células com DNA fragmentado. Porém, após cultivo em meio contendo FBS 10%, G30 aumentou a fragmentação de DNA. A presença do AM251 reverteu parcialmente ambos os efeitos.

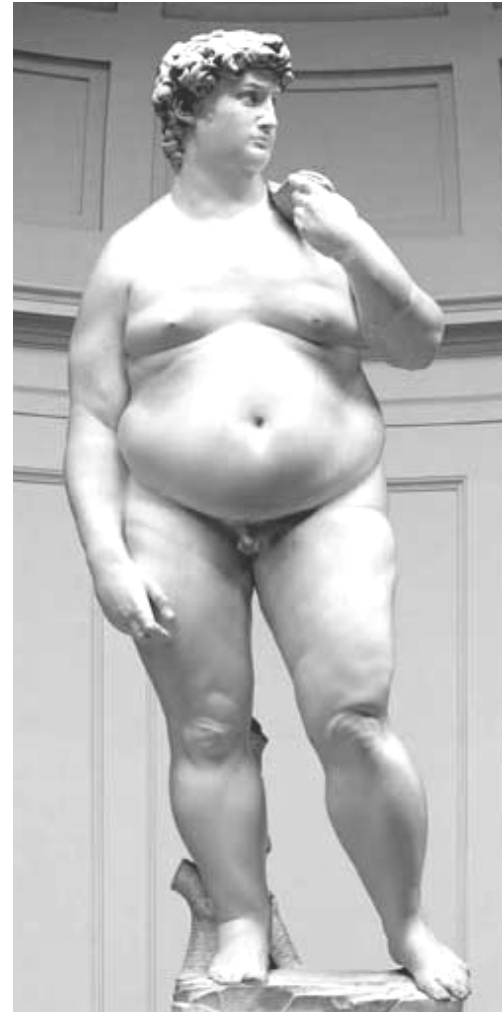
CONCLUSÕES

1. O antagonista CB1 têm um papel importante na regulação da sobrevivência da célula B pancreática. Quando as células são cultivadas em meio privado de soro o AM251 reverte o efeito protetor da glicose. Entretanto, em situações fisiológicas (presença de FBS) o antagonista CB1 protege a célula B da apoptose induzida por alta concentração de glicose.
2. O aumento na expressão de proteínas da via da UPR (ATF4 e CHOP) e o aumento no *splicing* do XBP1 pode ser o mecanismo pelo qual alta glicose protege da apoptose as células privadas de soro.

**Com Acomplia®
(Rimonabant)**



**Sem Acomplia®
(Rimonabant)**



AGRADECIMENTOS

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José Edgar Carvalho Nicoletti
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Jorge Vinícius Cestari Félix
José Luiz dos Santos
Fernando Forato Anhê
Tatiana S. Yamanaka
Adriana R. Leite

Prof^o José Cipolla Neto

Prof^a Andréa Torrão

Prof^o Rui Curi

Alice Cristina Rodrigues
Thaís Martins de Lima Salgado

Prof^o Angelo Carpinelli

Eduardo Rebelato

Prof^a Carla R. O. Carvalho