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MUSTAFA MUNIR MUSTAFA DAHLEH

**VERDADEIRAMENTE SELETIVO? YK11 E SEU IMPACTO NA DISFUNÇÃO
HIPOCAMPAL NO CONTEXTO DA MODULAÇÃO ANDROGÊNICA E
EXERCÍCIO**

Uruguiana

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Dissertação apresentada ao Programa de Pós-Graduação em Bioquímica da Universidade Federal do Pampa como requisito parcial para obtenção do título de Mestre em Bioquímica.

Orientadora: Marina Prigol

Coorientador: Gustavo Petri Guerra

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APRESENTAÇÃO

Esta dissertação é composta pelas seções de INTRODUÇÃO e REVISÃO BIBLIOGRÁFICA, que oferecem um panorama sobre o tema abordado, seguidas pela JUSTIFICATIVA do estudo e pelos OBJETIVOS propostos. A metodologia empregada e os resultados obtidos são apresentados na forma de artigos científicos previamente publicados, os quais estão incluídos na seção de RESULTADOS. Após a apresentação dos artigos, encontra-se a DISCUSSÃO e as CONCLUSÕES da dissertação, onde são apresentadas interpretações gerais sobre as pesquisas realizadas e as PERSPECTIVAS FUTURAS. Por último, as REFERÊNCIAS BIBLIOGRÁFICAS listam todas as referências citadas na dissertação, exceto as do item RESULTADOS, a quais já constam nos próprios artigos científicos.

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“Discipline equals freedom”

- John Gretton Willink Jr.

RESUMO

Esta dissertação investiga os efeitos neuroquímicos do modulador seletivo do receptor androgênico (SARM) YK11 [(17 α ,20E)-17,20-((1-metoxietileno)bis(óxido))-3-oxo-19-norpregna-4,20-dieno-21-carboxílico ácido metílico], administrado em doses conhecidas por exercer efeito anabólico, em combinação com exercício (EXE), com foco na análise do hipocampo de ratos machos. O YK11 vem ganhando popularidade entre atletas em busca de aprimoramento de desempenho, principalmente por sua capacidade de promover o crescimento muscular através da inibição da miostatina, um regulador chave da hipertrofia fisiológica. As análises *in silico*, incluindo ancoragem molecular e dinâmica molecular, demonstraram uma alta afinidade do YK11 com o receptor androgênico (AR), ressaltando interações significativas com a Arg752. Além disso, o YK11 apresentou elevado potencial inibitório da 5-alfa-redutase tipo II (5 α RII) principalmente através de interações com Arg114, o que sugere a inibição de biotransformações que poderiam limitar sua eficácia, aumentando seu efeito agonista no AR. A modelagem farmacocinética baseada em fisiologia (PBPK) revelou alta permeabilidade do YK11 ao sistema nervoso central (SNC), o que motivou a investigação de seus efeitos no hipocampo, região crítica para memória e aprendizagem, que possui um elevado número de AR, com alta afinidade por substâncias androgênicas. Estudos *in vivo* foram realizadas com ratos Wistar machos (200-250 g) durante 5 semanas, divididos em quatro grupos: Controle, YK11 (0,35 g/kg), EXE (natação) e EXE+YK11. Os resultados *ex vivo* indicaram que a administração de YK11 causou alterações significativas nas defesas antioxidante, com redução na atividade de enzimas antioxidantes e nos níveis de Nrf2. Essas alterações aumentaram a proteotoxicidade mediada pela Hsp70, gerando o aumento de parâmetros de estresse oxidativo, prejudicando o metabolismo mitocondrial no hipocampo, com diminuição da viabilidade celular e redução das proteínas Mn/SOD2, cruciais para a função mitocondrial. Além disso, em ratos que receberam YK11, foi observado uma diminuição nos níveis de subunidades da cadeia respiratória mitocondrial, como NDUFB8 (complexo I), CYTCB (complexo III) e ATP5A1 (ATP-sintase), bem como proteínas da dinâmica mitocondrial, incluindo Mfn1/2 e Drp1. Embora a combinação de EXE+YK11 tenha reduzido parcialmente alguns marcadores de função mitocondrial, não conseguiu reverter completamente os efeitos do YK11, como o aumento do estresse oxidativo e a disfunção das subunidades da cadeia respiratória mitocondrial e dinâmica mitocondrial. Ratos que receberam YK11 tiveram um aumento as citocinas pró-inflamatórias IL-1 β e IL-6 no hipocampo e reduziu a citocina anti-inflamatória IL-10, enquanto que o grupo EXE+YK11 apresentou elevação nos níveis de IL-10. Em relação as proteínas apoptóticas, observou-se aumento da p38 MAPK, desencadeando uma cascata apoptótica com

envolvimento de Bax/Bcl-2/caspase-3 clivada. Os ratos do grupo de EXE+YK11 demonstraram uma atenuação significativa nessas alterações, mas os níveis de Bax permaneceram elevados, sugerindo que o efeito protetor do exercício deve ser interpretado com cautela. Por fim, observou-se que tanto o grupo YK11 quanto o YK11 associado ao exercício apresentaram um comprometimento na consolidação da memória, modificando respostas de memória aversiva, mediada por alterações na via de neuroplasticidade pro-BDNF/BDNF/TrkB/CREB. Este estudo é pioneiro ao demonstrar os efeitos dos SARMs no sistema nervoso central, com foco específico no impacto do YK11 no hipocampo. Os resultados levantam preocupações significativas sobre os riscos neurológicos associados ao uso dessas substâncias por atletas, tanto profissionais quanto amadores, ao evidenciar uma elevada neurotoxicidade do YK11 em roedores.

Palavras-chave: SARM; Receptor Androgênico; Hipocampo; Neurotoxicidade; Exercício físico.

ABSTRACT

This dissertation investigates the neurochemical effects of the selective androgen receptor modulator (SARM) YK11 [(17 α ,20E)-17,20-((1-methoxyethylidene)bis(oxy))-3-oxo-19-norpregna-4,20-dien-21-oic acid methyl ester], administered at doses known to exert anabolic effects, in combination with exercise (EXE), focusing on hippocampal analysis in male rats. YK11 has gained popularity among athletes seeking performance enhancement, primarily due to its ability to promote muscle growth by inhibiting myostatin, a key regulator of physiological hypertrophy. *In silico* analyses, including molecular docking and molecular dynamics simulations, revealed a high binding affinity of YK11 to the androgen receptor (AR), highlighting significant interactions with Arg752. Furthermore, YK11 exhibited potent inhibitory activity against type II 5- α -reductase (5 α RII), primarily through interactions with Arg114, suggesting the inhibition of biotransformations that might limit its efficacy, thereby enhancing its agonistic effect on AR. Physiologically based pharmacokinetic (PBPK) modeling indicated high permeability of YK11 into the central nervous system (CNS), prompting an investigation of its effects on the hippocampus, a critical region for memory and learning that harbors a high density of ARs with strong affinity for androgenic compounds. *In vivo* studies were conducted using male Wistar rats (200–250 g) over a 5-week period, divided into four groups: Control, YK11 (0.35 g/kg), EXE (swimming), and EXE+YK11. *Ex vivo* results demonstrated that YK11 administration significantly altered antioxidant defenses, leading to reduced activity of antioxidant enzymes and decreased Nrf2 levels. These changes heightened proteotoxicity mediated by Hsp70, resulting in increased oxidative stress parameters and impaired mitochondrial metabolism in the hippocampus. Notably, there was a reduction in cell viability and diminished levels of Mn/SOD2 proteins, which are crucial for mitochondrial function. In addition, rats treated with YK11 exhibited decreased levels of mitochondrial respiratory chain subunits, including NDUF8 (complex I), CYTCB (complex III), and ATP5A1 (ATP synthase), alongside proteins involved in mitochondrial dynamics, such as Mfn1/2 and Drp1. Although the EXE+YK11 combination partially mitigated some markers of mitochondrial function, it failed to fully reverse YK11-induced effects, such as increased oxidative stress and dysfunction of mitochondrial respiratory chain subunits and dynamics. YK11 administration also led to elevated hippocampal levels of pro-inflammatory cytokines IL-1 β and IL-6, while reducing the anti-inflammatory cytokine IL-10. In contrast, the EXE+YK11 group showed increased IL-10 levels. Regarding apoptotic proteins, p38 MAPK activation was observed, triggering an apoptotic cascade involving Bax/Bcl-2/cI-Caspase-3. While the EXE+YK11 group demonstrated significant attenuation of these changes, Bax levels

remained elevated, suggesting that the protective effect of exercise should be interpreted cautiously. Finally, both the YK11 and EXE+YK11 groups exhibited impaired memory consolidation, altering aversive memory responses mediated by disruptions in the pro-BDNF/BDNF/TrkB/CREB neuroplasticity pathway. This study is the first to demonstrate the effects of SARMs on the CNS, with a specific focus on YK11's impact on the hippocampus. The findings raise significant concerns regarding the neurological risks associated with the use of these substances by both professional and amateur athletes, highlighting YK11 pronounced neurotoxicity in rodent models.

Keywords: SARM; Androgen Receptor; Hippocampus; Neurotoxicity; Exercise.

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AF1: Domínio de ativação 1, do inglês, Activation function 1, **AKT**: Proteína quinase B, do inglês, Protein kinase B, **AMPc**: Adenosina monofosfato cíclico, do inglês, Cyclic adenosine monophosphate, **AREs**: Elementos de Resposta Androgênica, do inglês, Androgen response elements, **AR**: Receptor androgênico, do inglês, Androgen receptor, **ATP5A1**: ATP sintase subunidade alfa 1, do inglês, ATP synthase subunit alpha 1, **Bax**: Proteína proapoptótica Bax, do inglês, Bcl-2-associated X protein, **Bcl-2**: Proteína antiapoptótica Bcl-2, do inglês, B-cell lymphoma 2, **BDNF**: Fator neurotrófico derivado do cérebro, do inglês, Brain-derived neurotrophic factor, **CAT**: Catalase, do inglês, Catalase, **CREB**: Proteína de ligação ao elemento de resposta ao AMPc, do inglês, cAMP response element-binding protein, **CYTCB**: Citocromo b, do inglês, Cytochrome b, **DBD**: Domínio de ligação ao DNA, do inglês, DNA-binding domain, **DHT**: Di-hidrotestosterona, do inglês, Dihydrotestosterone, **Drp1**: proteína do tipo dinamina-1, do inglês, Dynamin-related protein 1, **EAA**s: Esteroides anabólicos androgênicos, do inglês, Anabolic-androgenic steroids, **ERK**: Quinase regulada por sinais extracelulares, do inglês, Extracellular signal-regulated kinase, **ERs**: Receptores de estrogênio, do inglês, Estrogen receptors, **FKBP51**: A proteína 51 de ligação ao FK506, do inglês, FK506-binding protein 51, **Hsp**: Proteínas de choque térmico, do inglês, Heat shock proteins, **IGF-1**: Fator de crescimento semelhante à insulina tipo 1, do inglês, Insulin-like growth factor 1, **IL-1 β** : Interleucina-1 beta, do inglês, Interleukin-1 beta, **IL-2**: Interleucina-2, do inglês, Interleukin-2, **IL-6**: Interleucina-6, do inglês, Interleukin-6, **IL-10**: Interleucina-10, do inglês, Interleukin-10, **JAK**: Janus quinase, do inglês, Janus kinase, **LBD**: Domínio de ligação ao ligante, do inglês, Ligand-binding domain, **MAPK**: Quinase ativada por mitógeno, do inglês, Mitogen-activated protein kinase, **Mfn1**: Mitofusina 1, do inglês, Mitofusin 1, **Mfn2**: Mitofusina 2, do inglês, Mitofusin 2, **MRFs**: Fatores reguladores miogênicos, do inglês, Myogenic regulatory factors, **NDUFB8**: Subunidade B8 da NADH desidrogenase, do inglês, NADH dehydrogenase (ubiquinone) 1 beta subcomplex subunit 8, **NTD**: Domínio N-terminal, do inglês, N-terminal domain, **PGC1 α** : Coativador-1 alfa do receptor gama ativado por proliferadores de peroxissomos, do inglês, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, **PI3K**: Fosfatidilinositol 3-quinase, do inglês, Phosphoinositide 3-kinase, **PKA**: Proteína quinase A, do inglês, Protein kinase A, **p38 MAPK**: Quinase ativada por mitógeno p38, do inglês, p38 mitogen-activated protein kinase, **Progesterone receptor**: Receptor de progesterona, do inglês, Progesterone receptor, **SARM**: Modulador seletivo do receptor

androgênico, do inglês, Selective androgen receptor modulator, **STAT**: Transdutores de sinal e ativadores de transcrição, do inglês, Signal transducer and activator of transcription, **TNF- α** : Fator de necrose tumoral alfa, do inglês, Tumor necrosis factor alpha, **TNFR1**: Receptor 1 do fator de necrose tumoral, do inglês, Tumor necrosis factor receptor 1, **TrkB**: Receptor de tropomiosina quinase B, do inglês, Tropomyosin receptor kinase B.

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1. INTRODUÇÃO

Nos últimos anos, os moduladores seletivos de receptores androgênicos (SARMs) têm despertado crescente interesse devido ao seu potencial anabólico, especialmente quando combinados com exercícios físicos (MACHEK *et al.*, 2020; SOLOMON *et al.*, 2019). Esses compostos surgem como alternativas aos esteroides anabólicos androgênicos (EAAs) como testosterona, nandrolona e trembolona, prometendo efeitos similares sobre a musculatura esquelética, mas com menos efeitos colaterais (KHAN *et al.*, 2022; XIE *et al.*, 2022). Contudo, as semelhanças estruturais e os mecanismos de ação dos SARMs levantam preocupações sobre seu impacto no sistema nervoso central (SNC), uma vez que os EAAs estão associados a consequências neurodegenerativas significativas (HAUGER *et al.*, 2019; SESSA *et al.*, 2020; ZELLEROTH *et al.*, 2021). Por exemplo, Zelleroth *et al.* (2019) evidenciaram que substâncias como testosterona e trembolona ativam as caspases-3/7 e causam disfunções mitocondriais em células corticais de ratos, o que destaca a necessidade de investigar os riscos associados aos SARMs, visto que estes, apresentam estrutura química e mecanismo de ação similar aos EAAs.

Entre os SARMs, o YK11, conhecido quimicamente como 17 α ,20E)-17,20-[(1-Methoxyethylidene)bis(oxy)]-3-oxo-19-norpregna-4,20-diene-21-carboxylic acid methyl ester, destaca-se por ser um dos mais potentes desta classe (KANNO *et al.*, 2011). Este composto atua como agonista parcial do receptor androgênico (AR) e também como inibidor indireto da miostatina, promovendo uma marcante hipertrofia e hiperplasia muscular (KANNO *et al.*, 2011; KANNO *et al.*, 2013; LEE *et al.*, 2021). Apesar de seu potencial anabólico, o YK11 tem sido utilizado de forma indiscriminada por atletas que buscam melhorar o desempenho físico, mesmo sendo uma substância proibida pela Agência Mundial Antidoping (WADA) (WADA/USADA, 2020).

O uso indevido dessas substâncias por atletas representa uma crescente preocupação. A ingestão de doses elevadas, com o objetivo de amplificar os efeitos anabólicos, pode resultar em graves efeitos colaterais e riscos à saúde (VIGNALI *et al.*, 2023). Embora os impactos adversos dos EAAs sejam bem documentados, ainda existem muitas incertezas sobre os efeitos a longo prazo dos SARMs. Esse déficit de conhecimento é alarmante, principalmente em face da crescente popularidade desses compostos, cuja demanda global alcançou 115 milhões em 2021 (HAHAMYAN *et al.*, 2021). Além disso, o uso dos SARMs segue um padrão de aumento semelhante ao observado com os EAAs, com uma taxa de crescimento anual de aproximadamente 3,3% (VIGNALI *et al.*, 2023), sendo mais prevalente entre homens (PEREIRA *et al.*, 2019).

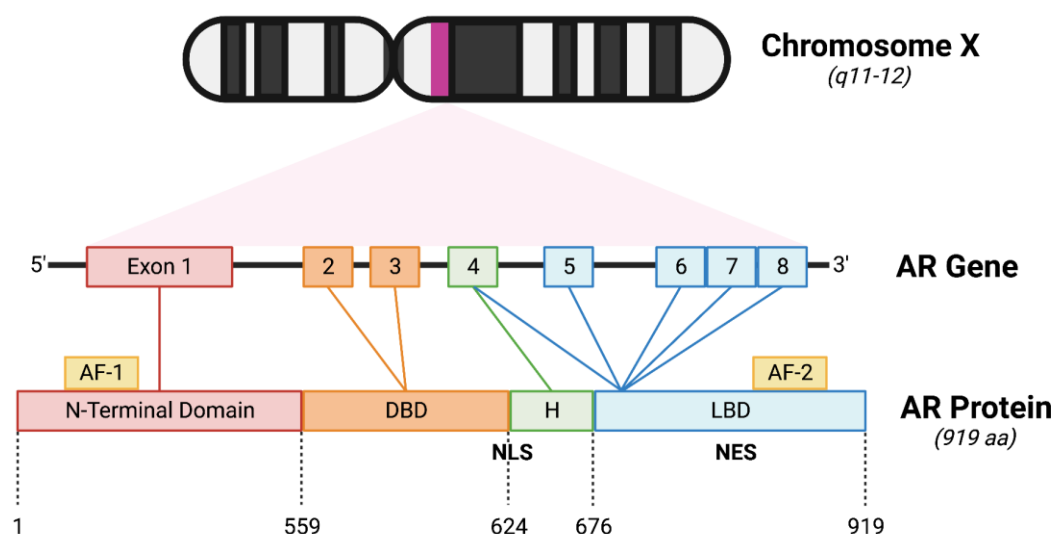
Apesar da sua ampla utilização, os efeitos dos SARMs sobre o SNC ainda são pouco explorados. Compreender os riscos potenciais associados a esses compostos é essencial para o estabelecimento de práticas seguras e diretrizes regulatórias adequadas. É particularmente importante investigar os possíveis efeitos neurotóxicos, que podem se assemelhar aos observados com o uso de esteroides anabolizantes (ZELLEROTH *et al.*, 2021; AGAHI *et al.*, 2022). Nesse contexto, nosso estudo visa investigar os mecanismos neuroquímicos potencialmente envolvidos no metabolismo do YK11 em doses anabólicas, associado ao exercício, buscando compreender seus efeitos no hipocampo, uma região fundamental para a função cognitiva e a consolidação da memória, que apresenta alta permeabilidade a substâncias androgênicas.

2. REFERENCIAL TEÓRICO

2.1. Receptor Androgênico (AR)

O AR, classificado como NR3C4 (do inglês, *nuclear receptor subfamily 3, group C, member 4*) (TOSO *et al.*, 2024), pertence ao grupo dos receptores de hormônios esteroidais, que inclui os receptores de progesterona (PR), glicocorticoides (GR) e estrogênio (ER) (TAN *et al.*, 2015; TOSO *et al.*, 2024). O gene que codifica o AR está localizado na região q11-q12 do cromossomo X e compreende uma sequência codificadora de 2757 nucleotídeos, dividida em oito exons e íntrons de tamanhos variados, entre 0,7 e 2,6 kb (Fig. 1) (CLINCKEMALIE *et al.*, 2012; TAN *et al.*, 2015). O AR é uma proteína composta por 919 aminoácidos, pesando aproximadamente 110 kDa, e estruturada em três domínios funcionais distintos: o domínio N-terminal (NTD), que abrange os resíduos de 1 a 559, o domínio de ligação ao DNA (DBD), localizado entre os resíduos 559 e 624, e o domínio de ligação ao ligante (LBD), situado entre os resíduos 676 e 919, conectado ao DBD por uma região flexível (resíduos 624 a 676) (Fig. 1) (OPPONG *et al.*, 2017; CHEN *et al.*, 2020).

Figura 1. Representação da estrutura dos domínios do AR.



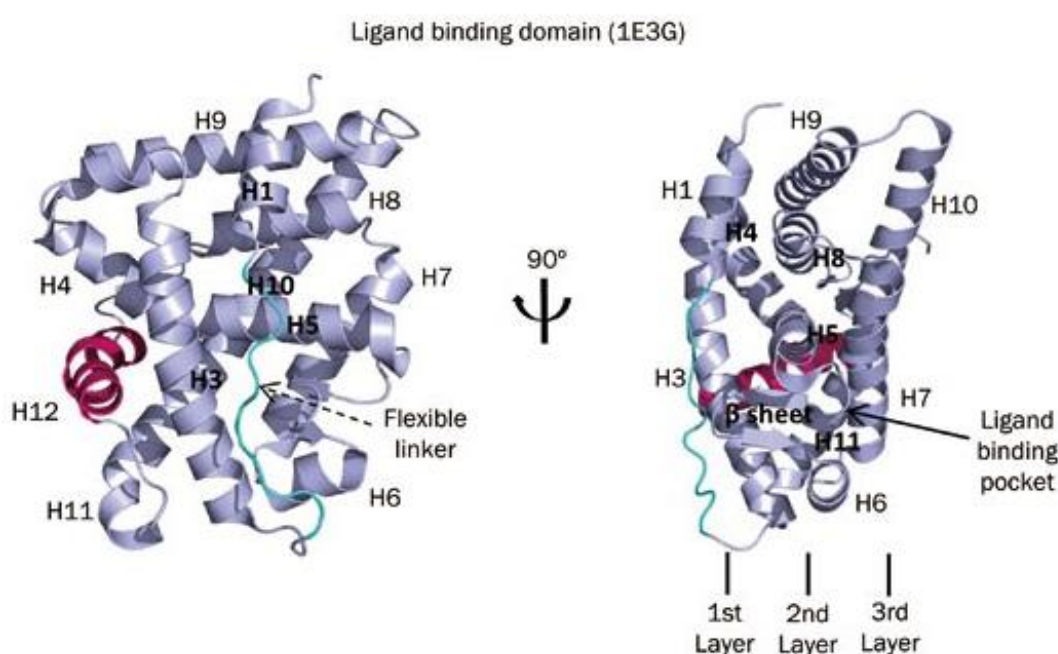
Fonte: Karen Parekh - © (2024) BioRender.

Cada um desses domínios desempenha papéis essenciais na função biológica do receptor. O NTD, responsável por processos de transativação, contém a função de ativação 1 (AF1), crucial para a regulação gênica mediada por andrógenos (KHAN *et al.*, 2024). Embora o NTD não participe diretamente das interações com o LBD, ele estabelece uma interface hidrofóbica com este, envolvendo motivos FXXLF, que são determinantes para a conformação e atividade geral do AR (DUBBINK *et al.*, 2004; DUBBINK *et al.*, 2006). O DBD, por sua vez, apresenta dois motivos de nucleases dedos de zinco, indispensáveis para a interação específica com o DNA nos Elementos de Resposta Androgênica (AREs) (XU *et al.*, 2023). A integridade estrutural do DBD é crítica para o alinhamento preciso do receptor, assegurando a interação eficiente com os outros domínios e com o DNA (OPPONG *et al.*, 2017; CHEN *et al.*, 2020; XU *et al.*, 2023).

O LBD é de particular importância por abrigar o resíduo Arg752, essencial para a estabilização de ligantes como a DHT e a testosterona (TAN *et al.*, 2015). O resíduo Arg752 forma ligações de hidrogênio com átomos polares dos ligantes, facilitando uma afinidade de ligação elevada (JESÚS-TRAN *et al.*, 2006; LEVINE *et al.*, 2014). Além disso, outros resíduos no LBD, como Asn705 e Gln711, contribuem para a estabilização do ligante e a subsequente ativação do receptor (JESÚS-TRAN *et al.*, 2006; LEVINE *et al.*, 2014; SAKKIAH *et al.*, 2018; SHIMMIN *et al.*, 2024). O LBD do AR é composto

por 11 hélices α e quatro curtas fitas β que formam duas folhas β antiparalelas (TAN *et al.*, 2015). Notavelmente, a hélice H2, presente em outros receptores nucleares, é substituída por uma longa região flexível no AR (TAN *et al.*, 2015; NADAL *et al.*, 2017). A ligação do ligante no LBD envolve uma reorganização conformacional, onde a hélice H12, que compõe o núcleo do domínio de ativação AF2, age como uma tampa que fecha o bolso de ligação ao ligante (LBP) após a interação com um agonista (Fig. 2) (TAN *et al.*, 2015).

Figura 2. Representação do domínio de ligação ao ligante (LBD) composto por 11 hélices α e duas pequenas folhas β -pregueada.

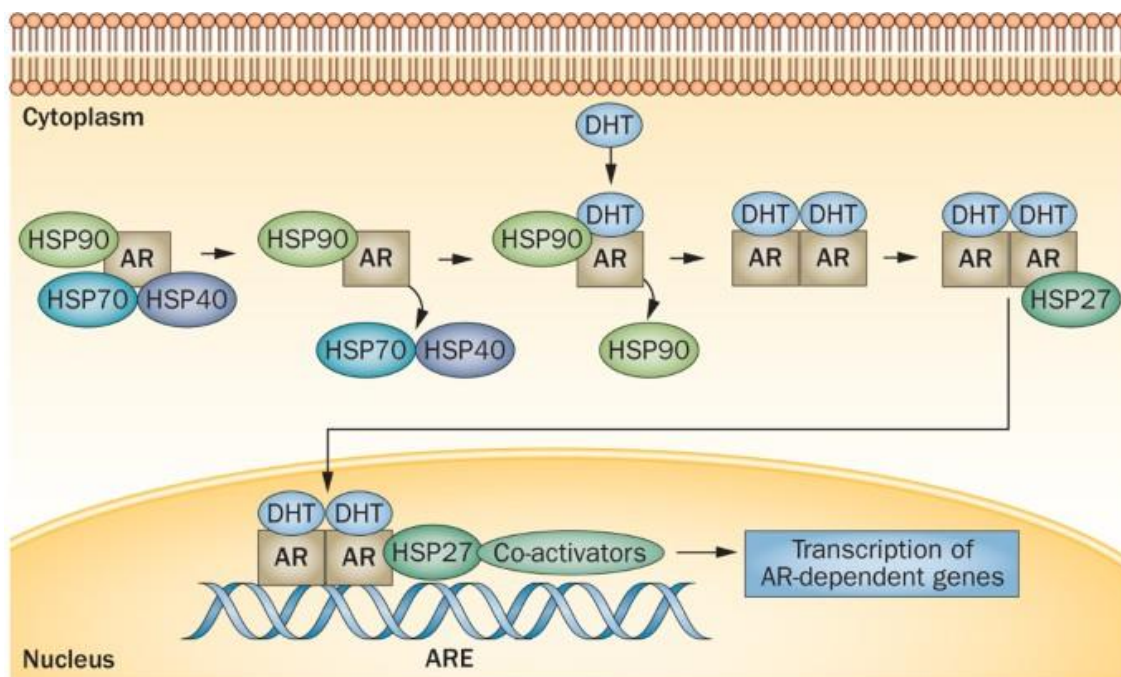


Fonte: TAN *et al.*, (2015).

Com o agonista, o processo de ativação do AR inicia-se com a difusão passiva de ligantes lipofílicos, como a testosterona e, principalmente, a DHT, através da membrana plasmática até o citoplasma, onde o AR reside em estado inativo, estabilizado por complexos proteicos, como as chaperonas Hsp90, Hsp70, Hsp40 (Fig. 3) (DAVEY AND GROSSMANN, 2016; EFTEKHARZADEH *et al.*, 2019). A interação do ligante com o LBD provoca uma mudança conformacional que libera o AR de suas proteínas chaperonas, permitindo a exposição de seus domínios funcionais e a preparação para a dimerização (Fig. 3) (DAVEY & GROSSMANN, 2016; EFTEKHARZADEH *et al.*, 2019). Esse rearranjo estrutural é mediado por interações entre os motivos FXXLF do NTD de um monômero de AR e o LBD, resultando na formação de homodímeros, onde

algumas frações ligam-se a Hsp27, que neste, não limitam a translocação nuclear (AZAD *et al.*, 2015).

Figura 3. O receptor androgênico (AR) envolvido em um complexo com chaperonas proteínas de choque térmico (da sigla Hsp, em inglês).



Fonte: AZAD *et al.*, (2015).

Uma vez dimerizado, o AR é translocado para o núcleo da célula, um processo facilitado por sequências de sinalização de localização nuclear presentes no receptor (Fig. 3) (AZAD *et al.*, 2015). No núcleo, os dímeros de AR ligam-se às sequências específicas de DNA, como nos AREs (EL KHARRAZ *et al.*, 2021). A ligação ao DNA é garantida pelos domínios de zinco no DBD, que interagem com alta especificidade com as regiões promotoras de genes-alvo regulados por andrógenos (LEE *et al.*, 2024). Após essa ligação, o AR recruta cofatores transcricionais, como o complexo p300/CBP e os coativadores da família SRC (EL KHARRAZ *et al.*, 2021; LEE *et al.*, 2024; TALUKDAR & CHATTERJI, 2023), que facilitam a modificação da cromatina e a acessibilidade da maquinaria transcricional. O recrutamento desses cofatores, junto com a fosforilação de resíduos-chave, resulta no recrutamento da RNA polimerase II e na ativação da transcrição de genes-alvo (TALUKDAR & CHATTERJI, 2023).

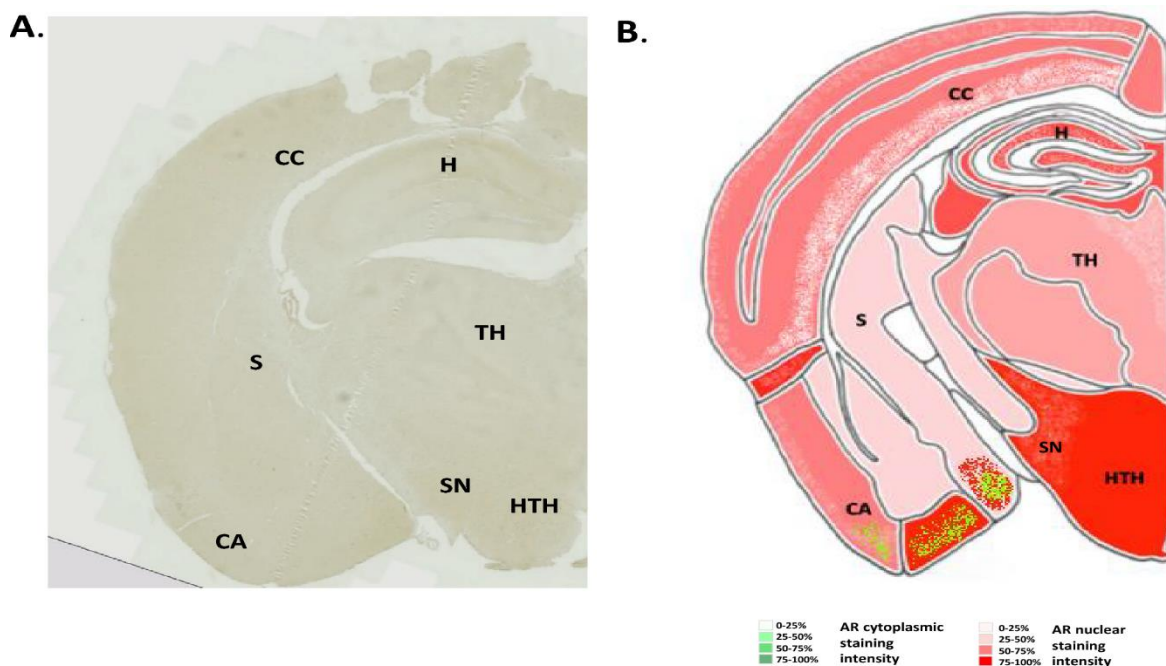
Esse complexo processo de ativação do AR culmina na transcrição de genes que promovem a diferenciação e proliferação celular, especialmente em tecidos dependentes

de andrógenos, como a próstata e o músculo esquelético (DAVEY AND GROSSMANN, 2016; AZAD *et al.*, 2015). Os produtos proteicos resultantes desempenham papéis essenciais na regulação do metabolismo celular e na resposta a estímulos androgênicos, influenciando tanto o crescimento celular quanto a homeostase tecidual (NADAL *et al.*, 2017). Entretanto, a ativação do AR é rigorosamente modulada por mecanismos de feedback negativo, que garantem sua temporária inatividade após a transcrição dos genes-alvo (EFTEKHARZADEH *et al.*, 2019). Entre esses mecanismos, destacam-se modificações pós-traducionais, como ubiquitinação, fosforilação e acetilação, que podem promover tanto a degradação quanto a reciclagem do receptor, interrompendo a sua atividade até que novos sinais hormonais sejam detectados, neste caso, comumente a sinalização de DHT (DAVEY AND GROSSMANN, 2016; AZAD *et al.*, 2015).

Adicionalmente, a afinidade do AR por seus dois principais ligantes endógenos, testosterona e DHT, apresenta sutis, mas significativas, diferenças que impactam a resposta biológica mediada pelo receptor (ORAFIDIYA *et al.*, 2018). Embora ambos os hormônios compartilhem uma estrutura similar, a ausência de uma ligação dupla entre os carbonos 4 e 5 no anel A da DHT altera sua geometria molecular, favorecendo interações mais fortes com o resíduo Arg752 do AR (JESÚS-TRAN *et al.*, 2006; LEVINE *et al.*, 2014). Isso confere à DHT uma maior afinidade de ligação, traduzindo-se em uma eficácia biológica maior em comparação à testosterona (JESÚS-TRAN *et al.*, 2006; LEVINE *et al.*, 2014; ORAFIDIYA *et al.*, 2018). Fisiologicamente, essas diferenças estruturais são refletidas na resposta androgênica, com a DHT desempenhando um papel mais central em processos como o desenvolvimento de características sexuais secundárias e a manutenção da homeostase de tecidos como a pele, músculos e o folículo piloso (ALEMANY, 2022). Dessa forma, a conversão da testosterona em DHT pela ação da enzima 5-alfa-redutase (5 α R) confere uma modulação mais refinada da resposta androgênica, aumentando a potência da sinalização em tecidos-alvo (HANDELSMAN, 2020; ALEMANY, 2022).

No cérebro, a ampla distribuição dos ARs desempenha um papel fundamental no desenvolvimento do SNC, sendo particularmente mais expressiva em machos, que apresentam uma maior densidade de ARs em comparação às fêmeas (DART *et al.*, 2024), além de uma sensibilidade mais elevada à modulação androgênica (FERNÁNDEZ-GUASTI *et al.*, 2022). Estudos recentes demonstram que, nos machos, o hipotálamo exibe a maior densidade de ARs nucleares, superando o hipocampo, o que está alinhado com sua função central no eixo regulador androgênico (Fig. 4) (DART *et al.*, 2024).

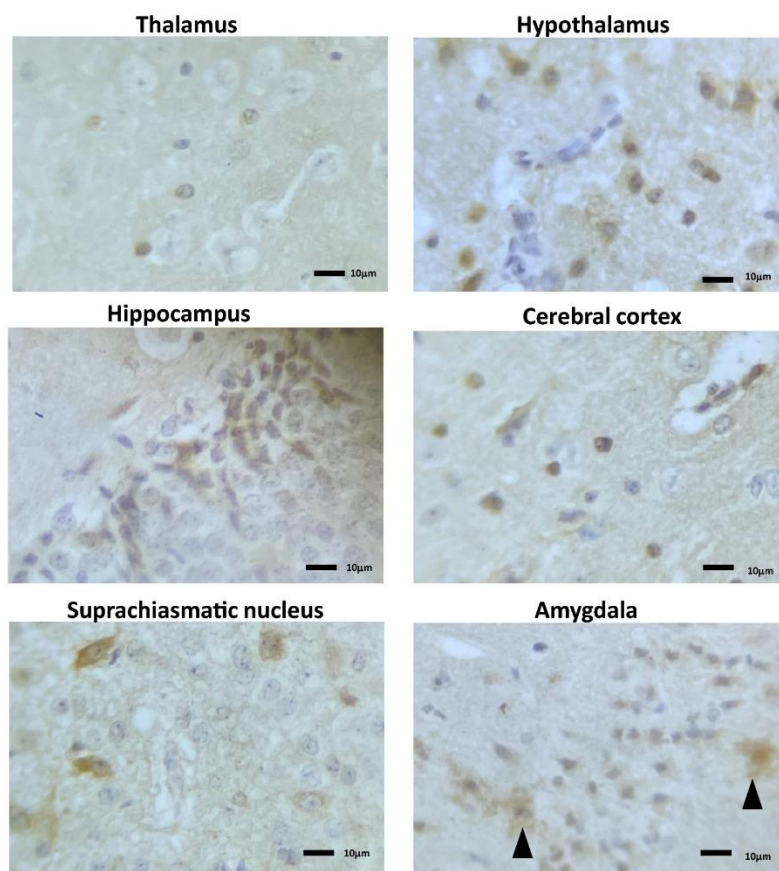
Figura 4. Distribuição de AR nas principais porções do SNC.



Fonte: DART et al., (2024).

No entanto, embora o hipotálamo apresente uma alta densidade de ARs nucleares, Dart *et al.* (2024) demonstram que o hipocampo possui uma densidade de ARs, relativa à área tecidual, que supera a do hipotálamo (Fig. 5), sugerindo uma resposta metabólica potencialmente mais robusta a estímulos androgênicos nessa região. A seguir, a área cortical amigdalal se destaca pela alta concentração de ARs nucleares, mas apresenta uma considerável densidade de ARs citoplasmáticos, o que limita a translocação para o núcleo e, consequentemente, a atividade transcricional mediada por andrógenos. Ademais, é importante ressaltar que Fernández-Guasti *et al.* (2022) evidenciam que os ARs hipocámpais, particularmente na sub-região CA3 de machos, são intensamente reativos aos estímulos androgênicos, reforçando a especialização dessa estrutura na resposta neuroquímica a hormônios esteroides. Complementando esses achados, Sato *et al.* (2004) e Chang *et al.* (2013) demonstram que a deleção de ARs em regiões específicas do cérebro resulta em uma redução significativa na regulação dos comportamentos sexuais. Nesse contexto, tanto o hipotálamo quanto o hipocampo exercem uma influência substancial na modulação inibitória desses comportamentos, revelando uma interdependência funcional crítica entre os ARs nucleares cerebrais e a efetiva ação androgênica.

Figura 5. Distribuição de AR nuclear nas principais porções do SNC.



Fonte: DART et al., (2024).

Com base nesse princípio, os Moduladores Seletivos do Receptor Androgênico (SARMs) surgem como uma promissora classe de compostos em desenvolvimento, designados para interagir seletivamente com o AR em tecidos específicos, como músculo esquelético e ósseo (SOLOMON *et al.*, 2019). Diferentemente dos andrógenos tradicionais, os SARMs são projetados para minimizar efeitos adversos em outros tecidos, como a próstata e SNC, potencialmente oferecendo uma alternativa terapêutica com menor risco de efeitos colaterais, mas mantendo a capacidade de promover o anabolismo muscular e a saúde óssea (NARAYANAN *et al.*, 2018). Assim, os SARMs representam uma abordagem inovadora para o tratamento de condições como sarcopenia e osteoporose, enquanto ainda aproveitam a robusta resposta biológica mediada pelo AR, principalmente derivada da característica de ligação mediada por DHT, demonstrada anteriormente.

2.2. Moduladores Seletivos dos Receptores Androgênicos (SARMs)

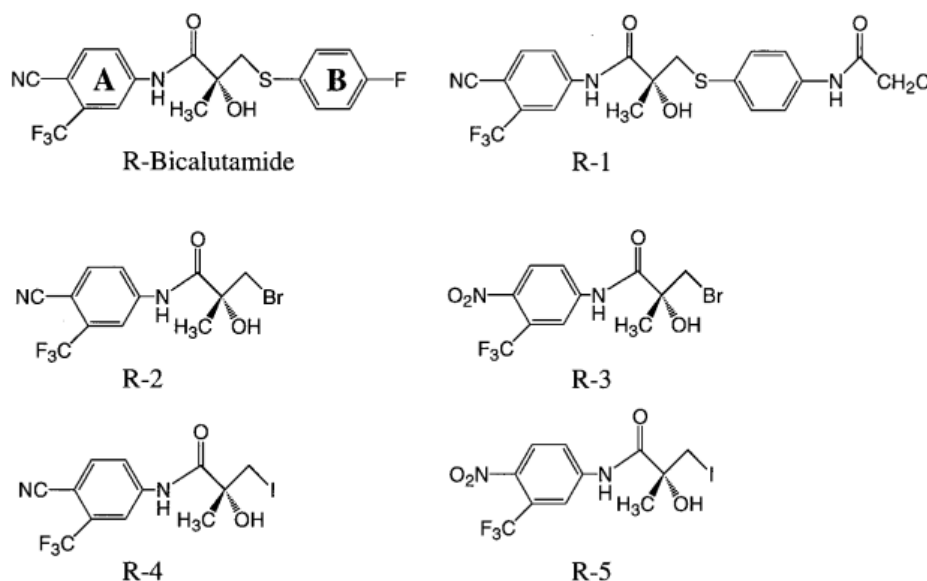
Os Moduladores Seletivos do Receptor Androgênico (SARMs, na sigla em inglês) são agonistas parciais do receptor androgênico, uma classe de fármacos desenvolvida com a intenção de apresentar alta seletividade pelo músculo estriado esquelético e pelo tecido ósseo (SOLOMON *et al.*, 2019). Os esforços iniciais para o desenvolvimento dos SARMs surgiram em paralelo ao crescente interesse por drogas sintéticas anabólicas, conhecidas como os EAAs, na década de 1940 (BHASIN & JASUJA, 2009). O uso de SARMs e EAAs, ainda produzidos clandestinamente, começou a ganhar atenção durante os Jogos Olímpicos de Verão de 1952, em Helsinque, onde foram detectadas substâncias derivadas da metandrostenolona em atletas vencedores de diversas modalidades esportivas (KREMENIK *et al.*, 2006; 2007; FITCH, 2008). Embora os métodos de detecção fossem pouco refinados, diversos exames indicaram a presença de substâncias androgênicas, como testosterona, DHT e 19-nortestosterona (nandrolona), além de traços metabólicos de compostos com características androgênicas indefinidas que mais tarde seriam identificados como SARMs (KREMENIK *et al.*, 2006; 2007; FITCH, 2008; BHASIN & JASUJA, 2009). Este período marca o início do desenvolvimento de novos EAAs e SARMs, ainda que não necessariamente sob essas designações (BHASIN & JASUJA, 2009).

Efetivamente, Dalton *et al.* 1998 publicou as primeiras versões que viriam a se tornar os SARMs, desenvolvidos como proposta terapêutica para fertilidade masculina e reposição e regulação do quadro hormonal masculino em indivíduos idosos. Estes primeiros compostos foram derivados da bicalutamida (IUPAC: N-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methylpropanamide), um antineoplásico com uma potente ação antiandrogênica e alta seletividade para o tecido prostático. A bicalutamida é composta por um núcleo benzotiazol, que contém átomos de enxofre e nitrogênio, proporcionando um caráter aromático heterocíclico, essencial para as interações da bicalutamida com o AR, garantindo sua função antiandrogênica (ASAAD & FILLERY, 2009). A presença de um grupo aril fluorado (um anel fenil trifluorado) confere à molécula maior estabilidade metabólica e alta lipofilicidade, favorecendo sua distribuição nos tecidos-alvo, particularmente na próstata (ASAAD & FILLERY, 2009; OSGUTHORPE & HAGLER, 2011). Além disso, a bicalutamida contém um grupo sulfonil ligado ao anel fenil, que é fundamental para a inibição da atividade de ligação de andrógenos, permitindo que a

molécula bloqueie eficazmente a sinalização androgênica (OSGUTHORPE & HAGLER, 2011; LIU *et al.*, 2015).

Essas características estruturais da bicalutamida foram exploradas para a criação de cinco isômeros distintos, denominados de R-1 a R-5 (Fig. 6), que mantêm o núcleo central da bicalutamida, mas variam em seus substituintes químicos para aumentar sua seletividade tecidual e otimizar o perfil de atividade dos compostos (DALTON *et al.*, 1998). A modificação seletiva dos grupos funcionais, como o posicionamento e a substituição de grupos fluorados, permitiu o desenvolvimento de compostos que mimetizam parcialmente a atividade androgênica, com o pressuposto de causar menores efeitos colaterais em comparação com os EAAs (THEVIS & SCHÄNZER, 2018; MACHEK *et al.*, 2020). De maneira geral, estas moléculas não tiveram aplicabilidade clínica, porém, tornaram-se fundamentais nos avanços tecnológicos no desenvolvimento destas substâncias.

Figura 6. Estrutura de ligantes não esteroidais do receptor de andrógenos baseados na estrutura do antiandrogênico bicalutamida.

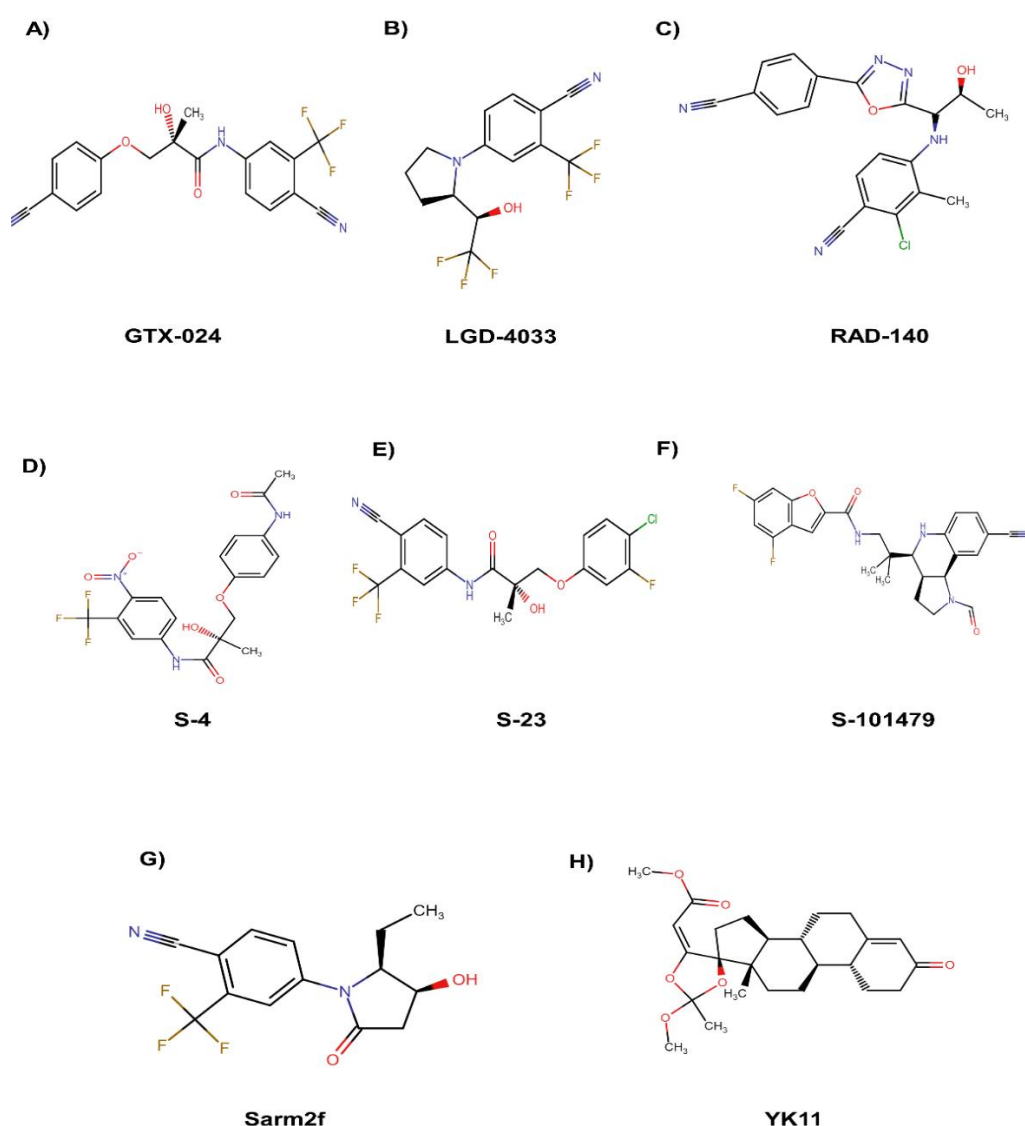


Fonte: DALTON *et al.*, (1998).

Na busca por ensaios pré-clínicos, Yin *et al.* (2003) e Gao *et al.* (2005) conduziram os primeiros testes sobre a ação dos SARMS, utilizando modelos baseados na estrutura da bicalutamida. Esses estudos demonstraram eficácia no aumento da hipertrofia em ratos machos castrados, com uma significativa seletividade ao receptor androgênico (AR). Entre as substâncias que foram ou estão sendo avaliadas em diversos

ensaios clínicos de fase II e III estão o Enobosarm (GTx-024, S-22; Fig. 8A) e o Ligandrol (LGD-4033, VK521; Fig. 8B), que têm indicações para tratamento de caquexia associada ao câncer, sarcopenia, câncer de mama e incontinência urinária de esforço (NARANAYAN *et al.*, 2018). Com o aumento da demanda por compostos com maior seletividade e menos efeitos adversos, diversos novos SARMs foram desenvolvidos ao longo do século XXI. Dentre eles, destacam-se a testolone (RAD-140; Fig. 8C), andarine (S-4; Fig. 8D), S-23 (Fig. 8E), S-101479 (Fig. 8F), Sarm2f (Fig. 8G) e YK11 (Fig. 7H), que estão atualmente em fase de testes.

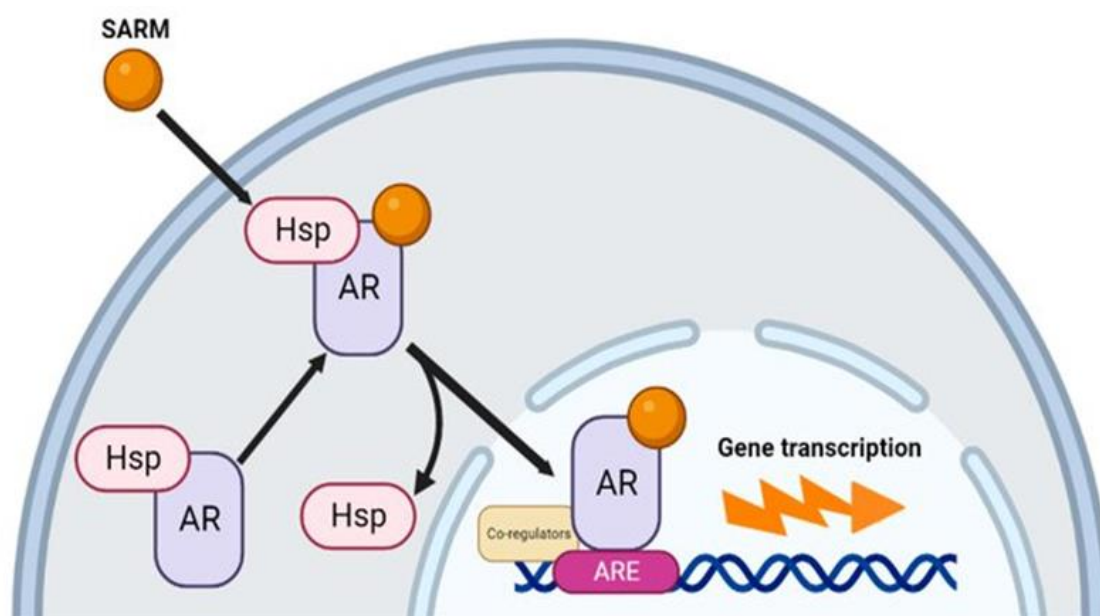
Figura 7. Principais SARMs em fase de testes pré-clínicos durante o século XXI



Fonte: Autor (2024).

De forma semelhante aos EAAs, os SARMs atuam principalmente através de sua ligação ao AR (MACHAK *et al.*, 2020). Esse processo promove um efeito anabólico ao causar a dissociação de proteínas de choque térmico (Hsp), o que resulta na translocação do complexo SARM-AR do citosol para o núcleo. No núcleo, ocorre a ligação de receptores androgênicos dimerizados aos AREs (MACHAK *et al.*, 2020; HALL & VROLIJK, 2023), ativando, assim, a expressão gênica que confere seu efeito androgênico (Fig. 8).

Figura 8. Mecanismo geral de ativação do AR e translocação do AR do citosol para o núcleo via ligação por SARMs.



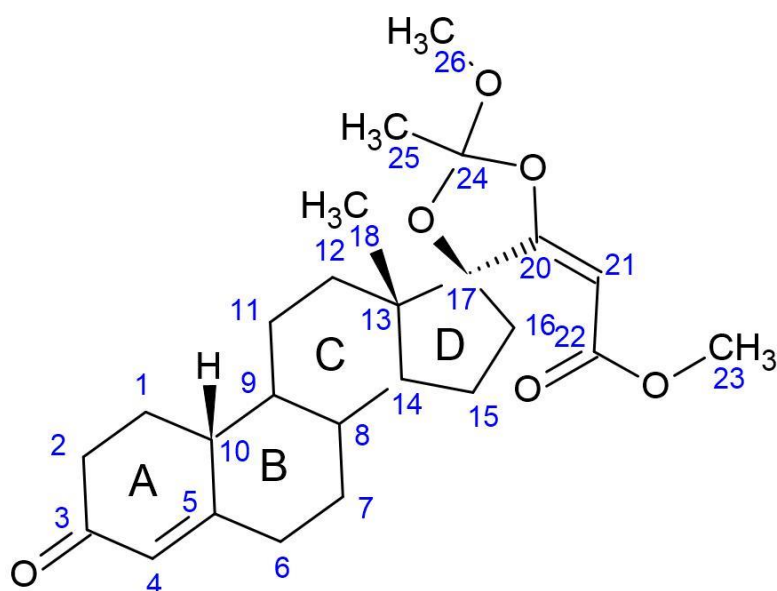
Fonte: HALL & VROLIJK, (2023).

2.2.1. Farmacologia do YK11

O éster metílico do ácido (17 α ,20E)-17,20-[(1-Metoxietilideno)bis(oxi)]-3-oxo-19-norpregna-4,20-dieno-21-carboxílico, mais conhecido como YK11 (Fig. 9), foi sintetizado por volta de 2011 como um agonista parcial do AR, conforme descrito por Kanno *et al.* (2011). Diferente da maioria dos SARMs, que geralmente possuem natureza não esteroidal, o YK11 destaca-se como uma das poucas exceções de estrutura esteroidal dentro desta classe, sendo derivado da acetilação de um agente progestogênico, a noretindrona (KATTO *et al.*, 2006; THEVIS & SCHÄNZER, 2018). Sua síntese envolve processos de ciclicização e carbonilação de ésteres propargílicos (KATTO *et al.*, 2006;

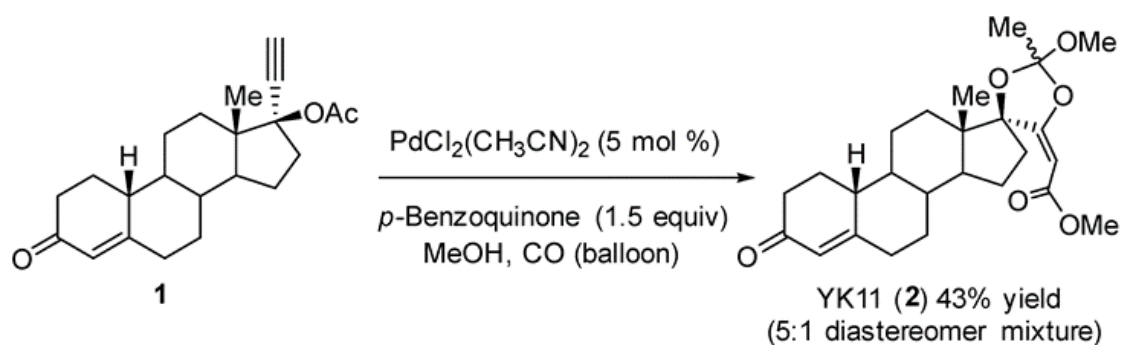
KANNO *et al.*, 2020), resultando em uma molécula que compartilha semelhanças estruturais com o esqueleto de carbono da DHT, mas com modificações que aumentam sua seletividade para no AR (KANNO *et al.*, 2011; 2013; 2020) (Fig. 10).

Figura 9. Estrutura química do éster metílico do Y11.



Fonte: Autor (2024).

Figura 10. Síntese do YK11 resultante da acetilação da noretisterona com ciclização e carbonilação de ésteres propargílicos.



Fonte: KANNO *et al.*, (2020).

A estrutura química do YK11 é composta por um núcleo esteroidal tetracíclico, apresentando um grupo 3-oxo no anel A, uma característica comum a muitos hormônios

esteroides que reforça sua atividade androgênica (KANNO *et al.*, 2020). Além disso, a modificação no grupo funcional do YK11, que possui uma dupla ligação na posição 20, introduz um grupo 1-metoxietilideno ligado a dois átomos de oxigênio, situado no anel D. Essa alteração é fundamental para modificar a afinidade do YK11 pelo AR, aumentando sua estabilidade, prolongando o tempo de interação (KANNO *et al.*, 2013; LEE *et al.*, 2021). O grupo carboxilato metílico no carbono 21 também desempenha um papel importante, modulando a lipofilicidade da molécula e aumentando sua estabilidade e biodisponibilidade (KANNO *et al.*, 2020).

Como agonista seletivo do AR, o YK11 compartilha semelhanças funcionais com o DHT, mas apresenta características próprias. Tanto o YK11 quanto o DHT promovem a translocação do AR para o núcleo, onde o receptor se liga aos AREs nas regiões promotoras de genes-alvo (KANNO *et al.*, 2011; AZAD *et al.*, 2015; HALL & VROLIJK, 2023). No caso do DHT, há uma indução da interação entre o domínio AF-2, localizado no domínio de ligação ao ligante (LBD), e o motivo FxxLF no domínio amino-terminal (NTD), interação essa que é essencial para a ativação de certos genes regulados pelo AR (KANNO *et al.*, 2011).

No entanto, o YK11 demonstra um comportamento distinto, onde ao contrário do DHT, o YK11 não ativa o domínio AF-2 na presença de DBD-LBD, o que sugere que ele não induz a interação N/C necessária para a transativação completa (KANNO *et al.*, 2011). Por outro lado, o DHT promove a transativação de genes quando o DBD-LBD é co-expressado com o NTD, comprovando a existência da interação N/C dependente de ligante, e com o YK11, essa interação não é observada e, além disso, ele inibe a interação N/C induzida pelo DHT (KANNO *et al.*, 2011; 2013). Ainda assim, o YK11 é capaz de ativar genes regulados pelo AR, como a proteína 51 de ligação ao FK506 (FKBP51), sugerindo a existência de um mecanismo alternativo de transativação (KANNO *et al.*, 2011). O NTD, especialmente o AF-1, parece desempenhar um papel essencial na ativação de genes mediada pela ligação com YK11 (KANNO *et al.*, 2011), o que confere a ele maior seletividade comparado aos EAAs, e similar ao DHT. Essa seletividade é uma característica dos SARMs, conhecidos por promoverem efeitos anabólicos em tecidos como músculos e ossos, sem causar efeitos adversos em outros tecidos reprodutivos (MACHAK *et al.*, 2020).

O complexo YK11-AR tem como principal função estimular genes como o Fst, o que resulta em um aumento significativo da síntese de folistatina, promovendo uma intensa resposta de diferenciação miogênica no tecido muscular (KANNO *et al.*, 2020;

LEE *et al.*, 2021). A ativação dos genes MyoD e Myf5, reguladores fundamentais dos fatores reguladores miogênicos (MRFs), é mais potente via YK11 do que via DHT (KANNO *et al.*, 2013), o que evidencia o alto potencial anabólico do YK11. Altos níveis de folistatina, por sua vez, inibem a miostatina, uma proteína que limita o crescimento muscular, favorecendo o aumento da hipertrofia e hiperplasia das fibras musculares (CARRARELLI *et al.*, 2015; LEGÅRD & PEDERSEN, 2019; SKRZYPCZAK *et al.*, 2021). Assim, o YK11 destaca-se não só pela sua seletividade similar ao DHT no AR, mas também por sua ação indireta na inibição da miostatina, sendo utilizada em doses elevadas, justamente buscando efeitos de inibição parcial dessa proteína (KANNO *et al.*, 2011).

2.3. Exercício e sua relação com compostos ergogênicos

Ao longo da história, o exercício físico tem sido fundamental para a saúde e o desempenho físico (HARGREAVES, 2021). Desde os tempos antigos, como os treinamentos militares na Grécia e as Olimpíadas, o exercício físico foi valorizado por seus efeitos na resistência corporal (TIPTON, 2014). Com o avanço científico, o exercício passou a ser estudado quanto aos seus efeitos modulatórios no organismo, surgindo então as práticas ergogênicas, que visam melhorar o desempenho físico (LÓPEZ-TORRES, 2022).

O exercício físico, amplamente reconhecido por seus efeitos neuroprotetores, desempenha um papel crucial na ativação de vias intracelulares que promovem a neuroplasticidade, a neurogênese e a sobrevivência neuronal (Lin *et al.*, 2018; Liu *et al.*, 2019). Um dos principais mecanismos envolvidos é o aumento na expressão do fator neurotrófico derivado do cérebro (BDNF), especialmente durante exercícios aeróbicos, que desempenha um papel central na modulação de processos cognitivos e na proteção contra danos neuronais (SLEIMAN *et al.*, 2016; CEFIS *et al.*, 2023). Ao se ligar ao receptor TrkB, o BDNF desencadeia a ativação de vias intracelulares, como MAPK/ERK e PI3K/Akt, fundamentais para a sobrevivência neuronal e a remodelação sináptica, particularmente no hipocampo, uma região-chave para a memória e o aprendizado (SLEIMAN *et al.*, 2016; CEFIS *et al.*, 2023). Adicionalmente, o exercício regula a via AMPc/PKA, promovendo a fosforilação de CREB, um fator de transcrição que estimula a expressão de genes associados à plasticidade sináptica e à consolidação da memória (Jin *et al.*, 2024). Esses mecanismos integrados potencializam a neuroplasticidade existente e estimulam a formação de novas sinapses, estabelecendo uma base robusta para a

adaptação cerebral a estímulos ambientais e ao estresse, além de conferir proteção contra processos neurodegenerativos (SAKAMOTO et al., 2011; LUO et al., 2017). Assim, o exercício físico, ao promover a liberação do BDNF e ativar vias moleculares associadas à plasticidade neuronal, consolida-se como uma estratégia essencial para a consolidando-se como uma estratégia essencial para a preservação das funções cognitivas e a mitigação de declínios associados ao envelhecimento ou a patologias neurológicas.

Substâncias androgênicas são frequentemente utilizadas com o objetivo de melhorar o desempenho esportivo. No entanto, o uso abusivo dessas substâncias pode comprometer os mecanismos neurobiológicos, interferindo nas cascatas bioquímicas essenciais para a manutenção da memória e da neuroproteção (Kenayama et al., 2013; Wood & Serpa, 2021; de Souza Penna et al., 2024). Essa interferência afeta de maneira negativa o hipocampo, uma região fundamental para os processos de aprendizado e memória (Lin et al., 2018; Liu et al., 2019), configurando-se como um ponto crucial de interesse em estudos que investigam a interação entre exercício físico e o uso dessas substâncias. No entanto, o exercício desempenha um papel crucial na regulação da sobrevivência celular, ativando o fator de crescimento semelhante à insulina 1 (IGF-1). Esse fator, por sua vez, ativa as vias PI3K/Akt e MAPK/ERK, resultando na inibição de proteínas pró-apoptóticas como a caspase-3, e aumenta a expressão de proteínas antiapoptóticas, como Bcl-2, promovendo assim um ambiente propício à sobrevivência neuronal (LIU *et al.*, 2021; LI *et al.*, 2023). Contudo, substâncias androgênicas podem reduzir a sinalização mediada pelo BDNF e pelo IGF-1 ao aumentar o estresse oxidativo, promovendo a apoptose neuronal (POMARA *et al.*, 2015). O uso prolongado de EAAs, por exemplo, aumenta a produção de espécies reativas de oxigênio (EROS), perturbando a regulação mitocondrial e exacerbando a apoptose ao inibir a via PI3K/Akt (DARBANDI *et al.*, 2018; BEKHET & EID, 2021).

Os SARMS, embora concebidos para exercer uma ação mais seletiva nos tecidos musculares, também possuem a capacidade de interagir com os ARs no cérebro (AKITA et al., 2013; JAYARAMAN et al., 2014). Essa interação pode resultar em uma ampla gama de efeitos metabólicos mediado pela ação nos ARs, considerando a significativa distribuição desses receptores no SNC (DART et al., 2024; FERNÁNDEZ-GUASTI et al., 2022). Essa interação pode resultar na ativação desregulada de vias de estresse oxidativo, levando a alterações mitocondriais e interferindo nas cascatas ativadas pelo exercício, como a via AMPc/PKA/CREB. Esse comprometimento pode prejudicar a consolidação da memória e atenuar os efeitos neuroprotetores associados ao exercício

físico (CHOI & LEE, 2011; NI *et al.*, 2024). Além disso, a influência de substâncias androgênicas pode reduzir a eficácia do exercício em proteger contra a apoptose, incapaz de inibir a caspase-3, uma enzima central na morte celular programada (ZELLEROTH *et al.*, 2019). Nesse sentido, as substâncias androgênicas podem comprometer a função mitocondrial no SNC, onde, enquanto o exercício estimula a biogênese mitocondrial por meio da ativação da proteína coativadora 1-alfa (PGC-1 α), melhorando o equilíbrio energético e a produção de antioxidantes intracelulares, o uso prolongado de androgênios poderá desregular essa função, resultando em aumento do estresse oxidativo e danos neuronais (DE OLIVEIRA BRISTOT *et al.*, 2019). Assim, a coadministração de SARMs com exercício, embora destinada a otimizar o desempenho físico, pode, paradoxalmente, comprometer os benefícios neuroprotetores associados à prática regular de atividades físicas.

3. JUSTIFICATIVA

A pesquisa sobre os SARMs, em particular o YK11, tem ganhado atenção crescente devido ao seu potencial para aumentar a massa muscular e reduzir a gordura, especialmente quando associado a programas regulares de exercício físico (Solomon *et al.*, 2019). O YK11 destaca-se como um potente SARM (KANNO *et al.*, 2011; 2013; 2020), funcionando como agonista parcial do AR e regulando proteínas específicas, como o aumento da folistatina, inibindo a ação da miostatina (LEE *et al.*, 2021). Esse aumento resulta na intensificação do desenvolvimento muscular e na regeneração do tecido ósseo (LEE *et al.*, 2021). Estudos recentes demonstram suas propriedades anabólicas, evidenciando sua capacidade de favorecer a proliferação e diferenciação de células precursoras de osteoblastos, como as MC3T3-E1 (YATSU *et al.*, 2018), e atuando como um importante regulador miogênico em células musculares C2C12, ao elevar os níveis de folistatina (KANNO *et al.*, 2013).

Contudo, persiste uma lacuna significativa na literatura sobre os efeitos do YK11 em outros sistemas, especialmente no SNC, com ênfase no hipocampo, um tecido com elevado resposta androgênica (JOKSIMOVIĆ *et al.*, 2017; 2019; Fernández-Guasti). Com o crescente interesse nos SARMs, surgem preocupações sobre a sua prevalência em populações específicas. Um estudo de HAHAMYAN *et al.* (2022) revelou um aumento expressivo de 115 milhões nas buscas relacionadas ao uso de SARMs até 2021, especialmente entre frequentadores de academias nos Países Baixos, onde o uso subiu 2,7%. O uso crescente do YK11, sem a devida regulamentação, é preocupante, dado o

desconhecimento sobre seus efeitos no SNC, um tecido altamente suscetível à toxicidade de derivados esteroidais androgênicos (SOLOMON *et al.*, 2019).

Diante desse cenário, é essencial investigar os efeitos do YK11 no hipocampo, particularmente quando utilizado em conjunto com o exercício, uma estratégia amplamente adotada para potencializar os efeitos anabólicos. Este estudo visa oferecer uma compreensão abrangente dos impactos neuroquímicos associados ao uso do YK11, e sua associação ao exercício, elucidando os principais mecanismos de sua ação no hipocampo. A escassez de pesquisas sobre o metabolismo e os potenciais efeitos adversos do YK11, incluindo sua neurotoxicidade, exige uma análise criteriosa e precavida quanto ao uso dessa substância. Para isso, essa dissertação adota uma abordagem integrada, utilizando modelos *in silico*, *in vivo* e *ex vivo*, permitindo uma convergência robusta de resultados sobre os efeitos primários do YK11 no SNC, com ênfase no hipocampo, uma área crítica para cognição e memória.

4. OBJETIVOS

4.1. Objetivo geral

Investigação dos efeitos neuroquímicos de YK11 combinado a exercício físico no hipocampo de ratos Wistar

4.2. Objetivos específicos

- Investigação do potencial de interação do YK11 com receptor androgênico e enzimas 5-alfa-redutase, além de sua distribuição tecidual em modelos de simulação computacional;
- Análise de parâmetros comportamentais de memória em ratos Wistar administrados com YK11 combinados a exercício físico;
- Avaliação do estresse oxidativo no hipocampo de ratos Wistar administrados com YK11 combinados a exercício físico;
- Avaliação das subunidades de complexos mitocondriais da cadeia respiratória no hipocampo de ratos Wistar administrados com YK11 combinados a exercício físico;
- Investigação da fusão e fissão mitocondrial no hipocampo de ratos Wistar administrados com YK11 combinados a exercício físico;
- Análise de mecanismos neurobiológicos de consolidação de memória no hipocampo de ratos Wistar administrados com YK11 combinados a exercício físico;

- Investigação da ativação de vias pró-apoptóticas no hipocampo de ratos Wistar administrados com YK11 combinados a exercício físico;
- Avaliação de mediadores inflamatórios no hipocampo de ratos Wistar administrados com YK11 combinados a exercício físico.

5. RESULTADOS

5.1. Artigo científico 1

ARTIGO: YK11 induces oxidative stress and mitochondrial dysfunction in hippocampus: The interplay between a selective androgen receptor modulator (SARM) and exercise

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YK11 induces oxidative stress and mitochondrial dysfunction in hippocampus: The interplay between a selective androgen receptor modulator (SARM) and exercise

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ABSTRACT

Our study investigates potential neurochemical effects of (17 α ,20E)–17,20-[(1-methoxyethylidene)bis(oxy)]–3-oxo-19-norpregna-4,20-diene-21-carboxylic acid methyl ester (YK11), a selective androgen receptor modulator (SARM), in the rat hippocampus, with a particular focus on oxidative stress and mitochondrial function, as well as its potential effect when combined with exercise (EXE). To validate YK11's anabolic potential, we performed a molecular docking analysis with the androgen receptor (AR), which showed high affinity with YK11, highlighting hydrogen interactions in Arg752. During the five-week protocol, we divided male Wistar rats into the following groups: Control, YK11 (0.35 g/kg), EXE (swimming protocol), and EXE+YK11. The administration of YK11 resulted in alterations in the endogenous antioxidant system, promoting increased oxidative stress and proteotoxic effects, impairing all mitochondrial function markers in the hippocampus. In contrast, EXE alone had a neuroprotective effect, increasing antioxidant defenses and improving mitochondrial metabolism. When combined, EXE+YK11 prevented alterations in some mitochondrial toxicity markers, including MnSOD/SOD2 and MTT reduction capacity, but did not reverse YK11's neurochemical impairments regarding increased oxidative stress and dysfunction of the mitochondrial respiratory chain and mitochondrial dynamics regulatory proteins in the hippocampus. In summary, our study identifies important pathways of YK11's hippocampal effects, revealing its potential to promote oxidative stress and mitochondrial dysfunction, suggesting that the administration of YK11 may pose potential neurological risks for athletes and bodybuilders seeking to enhance performance. These findings highlight the need for further research to assess the safety and efficacy of YK11 and SARM use in humans.

1. Introduction

The use of selective androgen receptor modulators (SARMs) has garnered attention in recent years due to their potential anabolic effects on muscle tissue, especially when combined with exercise [1,2]. These

compounds are purported to offer similar actions to anabolic-androgenic steroids (AAS) such as testosterone, nandrolone, and trenbolone, but without the associated adverse side effects [3]. However, the molecular similarity and mechanism of action of these compounds bring forth the perspective of their impact on the central nervous system (CNS), given

Abbreviations: AAS, Anabolic-Androgenic Steroids; ANOVA, Analysis of variance; AR, Androgen Receptor; CAT, Catalase; CYTB, Mitochondrially Encoded Cytochrome b; CYP, Cytochrome P450; DHT, Dihydrotestosterone; Drp1S637, Dynamin-related protein 1 serine 637; EXE, Exercise; FST, Follistatin; GPx, Glutathione Peroxidase; GR, Glutathione Reductase; GST, Glutathione S-Transferase; GSH, Reduced Glutathione; Hsp-70, Heat Shock Protein 70; Kcap-1, Kelch-like ECH-associated Protein 1; LG, Ligandrol; mTOR, Mammalian Target of Rapamycin; MnSOD/SOD2, Manganese Superoxide Dismutase; Mfn1, Mitofusin-1; Mfn2, Mitofusin-2; NDUFB8, NADH-ubiquinone oxidoreductase subunit B8; Nrf2, Nuclear Factor Erythroid 2-related Factor 2; OMM, Outer Mitochondrial Membrane; OS, Oxidative stress; RMSD, Root-Mean-Square Deviation; SARM, Selective Androgen Receptor Modulator; S.E.M, Standard Error of the Mean; SOD, Superoxide Dismutase YK11 (17 α ,20E)–17,20-[(1-methoxyethylidene)bis(oxy)]–3-oxo-19-norpregna-4,20-diene-21-carboxylic acid methyl ester.

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the evidenced neurodegenerative consequences of AAS [2].

Multiple AAS compounds with similar anabolic effects can induce different neurological impairments [4,5]. Zeller et al. [6] demonstrate that substances like testosterone, trenbolone, and nandrolone cause the activation of caspase-3/7 (extrinsic pathway) and mitochondrial metabolism dysfunction (intrinsic pathway) in primary cultures of rat cortical cells, leading to the upregulation of cellular apoptosis signaling [6,7]. The use of these substances has significant impacts, particularly trenbolone, which can cause the development of cortical dysplasia, mediated by the reduction of beta tubulin III (Tubb3), in addition to reducing neurite outgrowth and neuronal plasticity [8]. Exploration of the effects of SARMs, particularly in the CNS, becomes important given the evidence of these effects by AAS.

The class of SARMs includes several compounds, such as ostarine (enobosarm, S-22, MK-2866 or GTX-024), ligandrol (LGD-4033, VK5211), (17 α ,20E)–17,20-[(1-Methoxyethylidene)bis(oxy)]–3-oxo-19-norpregna-4,20-diene-21-carboxylic acid methyl ester (YK11), and RAD140 [1–3], highlighted by their effects on muscle tissue, resulting in increased body weight, hypertrophy, and hyperplasia of myoblasts, with greater vascularization of muscle fiber capillaries [9–11]. Despite the anabolic effects of these compounds, there have been limited studies on their neuronal effects. RAD140 is one SARM that has been studied and demonstrated to reduce extracellular signal-regulated kinase 1/2 (ERK) activity in the rat hippocampus, resulting in increased neuronal survival [12]. To overcome this limitation, it is crucial to investigate the impact of SARMs on the CNS, particularly those that possess higher anabolic potential, similar to trenbolone in AAS, which has been linked to significant neurotoxicity in the hippocampus, apparently the most affected tissue by AAS in the CNS [4,6,8].

Due to the emerging nature of SARMs, there is limited available data on their usage. However, a study conducted by HAHAMYAN et al. [13] revealed an increase of 115 million in global searches for these substances by 2021. This surge in interest resulted in a 2.7% rise in the utilization of these compounds across different regions in the Netherlands, particularly among individuals attending training centers [13]. Currently, the prevalence of SARM use is unknown, but according to Vignali et al. [14], their use is estimated to align with the trend of AAS, with a yearly increase of 3.3% in the utilization of androgenic substances worldwide, both male and female athletes. Consequently, the World Anti-Doping Agency (WADA) banned SARMs in the professional athletic scene [15]. However, there was still an observed rise in positive detections of these substances among athletes from various sports between 2015 and 2019 [15], including the use of YK11 [1].

YK11 stands out as a mighty SARM [16,17], working as a partial agonist of the androgen receptor (AR) by targeting specific genes, such as myostatin (GDF8), by increasing the synthesis of follistatin (FST), enhancing its potential in promoting muscle growth and bone tissue development [10]. YK11 has been extensively researched for its anabolic properties in the literature, with studies demonstrating its ability to enhance the proliferation and differentiation of MC3T3-E1 cells, a precursor cell line of osteoblasts [17], as well as acting as a myogenic regulator of C2C12 cells by increasing FST levels in the muscle [10]. Despite these findings, there is currently a lack of literature examining the effects of YK11 on other systems, such as the CNS, specifically the hippocampus.

Considering the aforementioned factors, the aim of this study is to examine the impact of YK11 on neurochemical parameters in the rat hippocampus, with a particular emphasis on oxidative stress and mitochondrial function, as well as its potential effect combined with exercise.

2. Materials and Methods

2.1. Chemicals

3,3',5,5'-Tetramethylbenzidine (TMB), potassium chloride (KCl), magnesium chloride (MgCl₂), ethylenediaminetetraacetic acid (EDTA),

sodium fluoride (NaF), aprotinin, β -glycerolphosphate, phenylmethylsulfonyl fluoride (PMSF), dithiothreitol (DTT), sodium orthovanadate, HEPES, sodium chloride (NaCl), glycerol, reduced glutathione (GSH), and bromophenol blue was purchased from Sigma-Aldrich® (St. Louis, MO, USA). YK11 and other chemicals were obtained of analytical grade or from standard commercial suppliers.

2.2. Animals

Male Wistar rats (200–250 g; 60-days-old) were obtained from the Central Biotery of the Federal University of Santa Maria (Rio Grande do Sul, Brazil). Twenty-four rats were used for the experimental design. The animals were housed in acrylic boxes in an air-conditioned room (22 $\pm$ 2 °C) with free access to food and water in a 12:12 h light/dark cycle (lights on at 7:00 a.m.). All experiments reported in this study were conducted in accordance with the National and International legislation guidelines of the Brazilian Council of Animal Experimentation (CONCEA), the U.S. Public Health Service's Policy on Humane Care and Use of Laboratory Animals (PHS Policy), and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and approved by local Ethics Committee for Animal Research of the Federal University of Pampa (CEUA protocol n° 009/2022).

2.3. Experimental design

Sixty-days-old Wistar rats were randomly selected into the following groups: control [vehicle + no YK11 + no exercise (EXE)], YK11 [vehicle + YK11 0.35 g/kg + no EXE], EXE [vehicle + no YK11 + EXE], and EXE+YK11 [vehicle + YK11 0.35 g/kg + EXE]. The administration of vehicle solution (1 mL/kg 1% ethanol dissolved in 0.9% saline) or YK11 (0.35 g/kg in 1% ethanol dissolved in 0.9% saline) was received by oral gavage, once a day, underwent in 5-week protocol (Fig. 1). The lowest recorded dose of YK11 (0.35 g/kg/day) in rodents [16] was utilized to replicate an abusive scenario, reflecting the escalating worldwide trend of SARMs usage [14]. All animals used to determine neurochemical parameters were tested for myostatin immunoreactivity in the gastrocnemius muscle (Fig. S1), in order to validate EXE and YK11 anabolic effect. The 5-week swimming exercise protocol induced was adapted from Stone et al. [16], based on a 0–2% of rat's body weight (BW) progressive-load protocol, 20–40 cm of water depth, 20–50 min, 5 days/week (Table 1). On the 46th day, two days before the last day of swimming protocol in order to minimize variables of physical fatigue, the animals were euthanized, and the hippocampus was rapidly removed for ex vivo analysis. Due to the ability of SARMs to pass the blood-brain barrier [12], acting mainly in hippocampus, similarly to AAS [4,6,8], this tissue was used to evaluate YK11 neurological effects.

2.4. In silico assays

2.4.1. Protein-ligand interactions potential of YK11 with AR in molecular docking

The crystal structure of YK11 (X-ray diffraction molecular configuration) was obtained from the Cambridge Crystallographic Data Center (CCDC; code: 2178474) [19]. Accuracy of the docking protocol was assessed by redocking 5 α -Dihydrotestosterone (DHT; PDB code: 4OEA; X-ray diffraction molecular configuration) native co-crystallized ligand binding with human AR (PDB code: 4OEA; X-ray diffraction molecular configuration) [20]. The docked positions were compared to the crystal structure positions by calculating RMSD (root-mean-square deviation) values. The AR-DHT native model was validated, showing a RMSD = 0.3301 Å (acceptance of $\leq$ 2 Å) (Fig. S2) [21]. The ligands were considered flexible (PM6 charges) and the receptors rigid (Gasteiger charges) in AutoDockTools [22]. Analyses of AR-YK11 interactions consisted in the deletion of the native DHT ligand, remaining AR chain A residue-specific binding free. For the docking simulations,

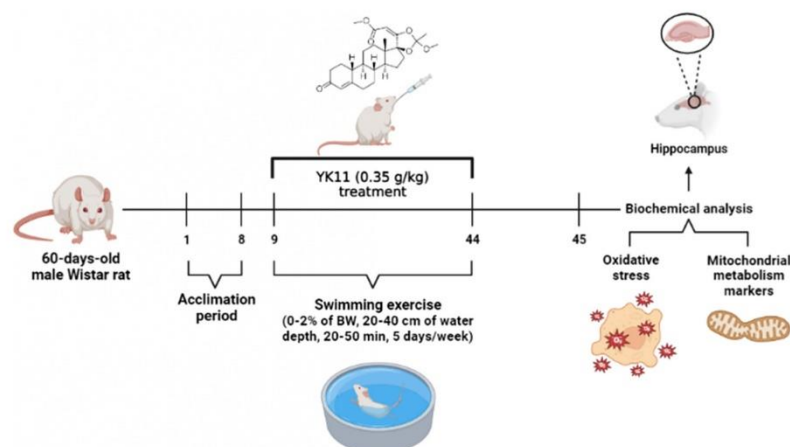


Fig. 1. Schematic representation of the experimental design.

Table 1
Summary of five-week swimming exercise protocol.

Week	Intensity (%) *	Water depth (cm)	Training time (min/day)
1	0	20	20
2	0	30	30
3	1	30	30
4	1	30	40
5	2	40	50

*Percentage of weight attached to a rat based on its body weight (BW).

AutoDockVina software [23] was used, with a gridbox that involved the entire protein structure ($74 \times 60 \times 48$) and the coordinates $x = -21.379$; $y = 2.452$; $z = -5145$. The exhaustiveness of 100 was used. Results were analyzed using AutoDockVina software and Discovery Studio Visualizer software.

2.5. Ex vivo assays

The hippocampal samples were homogenized in 50 mM Tris-HCl, pH 7.4 (1/5, w/v), and the resulting homogenate was centrifuged at 10,000g for 10 min at 4 °C. The supernatant fraction (S1) was used for biochemical assays, except for mitochondrial viability, GSH, and western blot analysis.

2.6. Thiobarbituric acid-reactive substances (TBARS) levels

Lipid peroxidation was determined by measuring TBARS levels as described by Ohkawa [24], based on the release of malondialdehyde (MDA), a product of fatty acid peroxidation which reacts with thiobarbituric acid (TBA). Briefly, 200 μ L of S1 was added to 500 μ L of 0.8% TBA, 200 μ L of 8.1% sodium dodecyl sulfate (SDS), and 500 μ L of acetic acid, with the mixture incubated at 95 °C for 2 h. The absorbance was measured at 532 nm, and the results were expressed as nmol MDA/mg protein.

2.7. Determination of reactive species (RS)

The estimation of RS production in the hippocampus was assayed according to Loetchutinat [25]. Briefly, 10 μ L of S1 was added to 3000 μ L of Tris HCl 10 mM pH 7.4, and the reaction was initiated with 1 mM of 2',7'-dichlorofluorescein diacetate (DCFH-DA), a

non-fluorescent compound that easily crosses cell membranes and is rapidly oxidized to dichlorofluorescein (DCF), a fluorescent derivative, in the presence of RS. The fluorescence emission of DCFH-DA was monitored after 1 h at an excitation wavelength of 488 nm and an emission wavelength of 520 nm using a fluorescence spectrometer. The results were obtained and expressed as a percentage of the control DCF formation in arbitrary units (AU).

2.8. Superoxide dismutase (SOD) activity

SOD activity was measured according to Misra and Fridovich [26], based on the ability of SOD to inhibit epinephrine oxidation via superoxide anion ($O_2^{\cdot -}$) to adrenochrome, resulting in the release of H_2O_2 . The reaction was initiated by adding aliquots of S1 (6–18 μ L) to 30 μ L of 6 mM epinephrine (substrate) in a medium containing aliquots (260–242 μ L) of 57.7 mM sodium carbonate buffer, 30 °C. The absorbance of the samples was read at 10-second intervals for 2 min using a spectrophotometer at 480 nm. One unit of enzyme was defined as the amount of enzyme required to inhibit the epinephrine oxidation by 50% at 30 °C. The results were expressed as U/mg protein.

2.9. Catalase (CAT) activity

CAT activity was measured according to the method described by Aebi [27], based on the clearance of H_2O_2 in the presence of S1 at 240 nm. The reaction was initiated by adding 20 μ L of S1 to 105 μ L of 0.3 mM H_2O_2 (substrate) in a medium containing 100 μ L of 50 mM phosphate buffer, pH 7.0. The results were expressed in Units, where one Unit decomposes 1 μ mole of H_2O_2 /minute at pH 7.0, 25 °C.

2.10. Total glutathione levels

Total GSH levels were determined using the method described by Hissin and Hilf [28]. The GSH levels were measured by homogenizing hippocampus in 0.1 M HCl₄ and centrifuging the homogenates at 2500g for 10 min at 4 °C. The resulting supernatants were utilized. A 100- μ L portion of the supernatant was incubated in the dark for 15 min with 800 μ L of TFK buffer 0.1 M pH 8.0 and 100 μ L of ortho-phthalaldehyde as a fluorophore. The samples were analyzed in a fluorescence spectrophotometer with an excitation wavelength of 350 nm and emission wavelength of 420 nm. The results were expressed as nmol GSH/mg tissue using the mean value.

2.11. Glutathione peroxidase (GPx) activity

GPx activity was measured spectrophotometrically, following the method described by Wendel [29], which involves oxidizing GSH to GSSG and dismutation of H_2O_2 in water. The GPx activity was indirectly measured by monitoring the decay of NADPH when added to the reaction, which undergoes the action of GR, forming GSH and the product $NADP^+$. In this assay, 800 μ L of reductase system (NADPH 0.15 mM, GSH 1 mM, 10 μ L of GR (500 U), and 250 μ L of azide 100 mM, dissolved in 20 mL of TFK buffer 100 mM pH 7.0), with 50 μ L of S1 and 100 μ L of H_2O_2 4 mM were added to the samples. The absorbance of the samples was read at 30-second intervals for 2 min using a spectrophotometer at 340 nm. The results were expressed as nmol NADPH/min/mg protein.

2.12. Glutathione reductase (GR) activity

GR activity was measured using the method described by Carlberg and Mannervik [30], which is based on the consumption of NADPH proportional to the GR activity. In this assay, 750 μ L of reductase system (0.15 M TFK/EDTA 1.5 mM pH 7.0 and NADPH 0.15 mM) with 100 μ L of S1 was added, and spectrophotometry was performed at a wavelength of 340 nm at 30-second intervals for 2 min. Afterward, 50 μ L of GSSG was added, and the spectrophotometer was used to analyze the wavelength of 340 nm at 30-second intervals for another 2 min. A duplicate of each sample was performed using the mean value. The difference between both absorbances was calculated, and the results were expressed as nmol NADPH/min/mg protein.

2.13. Glutathione S-transferase (GST) activity

GST activity was assayed by the conjugation of glutathione with 1-chloro-2,4-dinitrobenzene (CDNB), as described by Habig et al. [31]. GST is an important phase II enzyme involved in the detoxification of xenobiotic substances. To perform the assay, 50 μ L of S1 was mixed with 0.1 M potassium phosphate buffer (pH 7.5), 50 mM CDNB as a substrate, and 100 mM GSH to initiate the reaction. The absorbance of the samples was read at 30-second intervals for 2 min using a spectrophotometer at 340 nm. The results were expressed as μ mol/min/mg protein.

2.14. Mitochondrial viability

To evaluate mitochondrial viability in rat hippocampus, mitochondrial isolates were prepared using the method described by Mosmann [32] with slight modifications. Briefly, the tissue was homogenized in 1000 μ L of Tris-sucrose buffer (0.25 M, pH 7.4) and centrifuged at 1000g for 5 min at 4 °C. The enriched mitochondrial fraction was obtained through postnuclear supernatant centrifugation (10000g, 10 min, 4 °C), while the remaining pellet was washed with mannitol-sucrose-HEPES buffer and resuspended in 200 μ L of suspension buffer. Next, a 200 μ L portion of the mitochondrial fraction was incubated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (0.5 mg/mL) for 30 min at 37 °C. The samples were then centrifuged at 10000g for 5 min at 4 °C, and the pellet was incubated in dimethyl sulfoxide (DMSO) solution for 30 min at 37 °C. The absorbance was measured at 570 nm, and the results were expressed as percentage of the Control.

2.15. Western blot analysis

The western blot analysis was performed described by Guerra et al. [33]. For sample preparation, rats were decapitated, and the hippocampus was rapidly removed and homogenized in ice-cold buffer containing proteases and phosphatases inhibitor cocktail (Sigma-Aldrich®; St. Louis, MO, USA). Protein concentration was determined as described by Bradford [34], and all sample extracts were diluted to a final protein concentration of 80 μ g.

The proteins were separated using 12% SDS-PAGE gels for approximately 3 h. Subsequently, the gel was transferred onto Amersham™ Protran® Premium Western blotting nitrocellulose membranes, using the Transfer-Blot® Turbo™ Transfer System (1.0 mA; 30 min). To confirm protein transfer, the membranes were stained in ponceau 0.5% [35]. After being blocked for 2 h with 1% bovine serum albumin (BSA) in 0.05% Tween 20 in saline Tris-borate (TBS-T), the blots were incubated overnight with specific primary polyclonal antibodies anti-mouse β -actin (1:1000; Sigma-Aldrich®; St. Louis, MO, USA) served as a loading control, anti-rabbit Manganese-superoxide dismutase (MnSOD/SOD2) (1:1000; Sigma-Aldrich®; St. Louis, MO, USA), anti-mouse nuclear factor erythroid 2-related factor 2 (Nrf2) (1:1000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), anti-rabbit heat shock protein 70 (Hsp-70) (1:1000; Sigma-Aldrich®; St. Louis, MO, USA), anti-rabbit NADH-ubiquinone oxidoreductase subunit B8 (NDUFB8) (complex I) (1:1000; Sigma-Aldrich®; St. Louis, MO, USA), anti-rabbit mitochondrially encoded cytochrome b (CYTB) (complex III) (1:1000; Sigma-Aldrich®; St. Louis, MO, USA), ATP synthase F1 subunit alpha (ATP5A1) (complex V) (1:1000; Sigma-Aldrich®; St. Louis, MO, USA), anti-rabbit mitofusin-1 (Mfn1) (1:1000; Sigma-Aldrich®; St. Louis, MO, USA), anti-rabbit mitofusin-2 (Mfn2) (1:1000; Sigma-Aldrich®; St. Louis, MO, USA), and anti-rabbit Dynamin-related protein 1 serine 637 (Drp1^{S637}) (1:1000; Sigma-Aldrich®; St. Louis, MO, USA). The blots were washed three times with 0.05% TBS-T, followed by incubation with a secondary antibody conjugated with horseradish peroxidase (1:5000, anti-rabbit IgG or anti-mouse IgG-HRP; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) for 2 h at room temperature. They were then developed with 3,3',5,5'-Tetramethylbenzidine (TMB; Sigma-Aldrich®; St. Louis, MO, USA) and quantified using ImageJ (NIH, Bethesda, MD, USA). The results were normalized by arbitrarily setting the ratio between the protein band and β -actin loading control.

2.16. Statistical analysis

The Shapiro-Wilk test was used to perform the normality test, while the Bartlett and F tests were conducted to check for homoscedasticity of variances. A one-way analysis of variance (ANOVA) was carried out based on classic unifactorial method $Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$, where Y_{ij} represents the observed value, μ represents the overall mean, α_i represents the fixed effect of the i -th group (Control, YK11, EXE, or EXE+YK11) – μ , and ϵ_{ij} represents the random experimental error. Tukey's post-hoc test was performed, and significance between groups was considered when $p < 0.05$. The data is presented as mean and standard error of the mean (S.E.M).

3. Results

3.1. Molecular docking in the validation of YK11 anabolic mechanism

The YK11 anabolic potential is verified in biological interactions with AR, and for this, we use AR-DHT (AR native ligand; Fig. S3; A and B), to compare AR-YK11 (SARM; Fig. S3; C and D) interactions. From the validated DHT, its interaction possessed a predicted $\Delta G_{\text{bind}} = -8.4 \text{ kcal mol}^{-1}$ with AR chain A through protein-ligand interactions as hydrogen bonds with Thr877 and Arg752, including alkyl and π -alkyl interactions with Leu704, Phe764, and Leu873 (Fig. 2A; Table 2). A DHT-like interaction was verified in YK11, possessing a predicted $\Delta G_{\text{bind}} = -7.6 \text{ kcal mol}^{-1}$ with AR chain A through protein-ligand interactions as alkyl interactions with Arg752 and Ala748, including van der Waals interaction with Glu681 and Pro682 (Fig. 2B, Table 2).

3.2. Reactive species (RS) levels and lipid peroxidation

RS levels (Fig. 3A) was increased in YK11 compared to the control ($p < 0.0001$), EXE ($p < 0.0001$) and EXE+YK11 ($p = 0.0004$), while in EXE had a decrease compared to the control ($p = 0.0307$), and

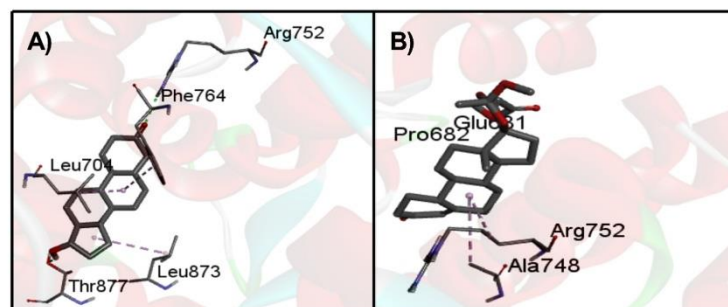


Fig. 2. Predicted interactions between AR with native DHT and YK11 on molecular docking simulations. Interactions with A) AR-DHT; B) AR-YK11. Data obtained using the software AutoDockVina and Discovery Studio Visualizer.

Table 2

Predicted ΔG_{bind} from native DHT and YK11 with AR on molecule docking studies.

Ligand	DHT	YK11
ΔG (kcal mol ⁻¹)	-8.4	-7.6

EXE+YK11 ($p = 0.0004$); EXE+YK11 has an increase compared to the control ($p = 0.0254$). Of note, EXE showed an average of $\approx 48\%$ of RS levels production (below 50% capacity). Lipid peroxidation (Fig. 3B) was increased in YK11 regarding control ($p < 0.0001$), EXE ($p < 0.0001$) and EXE+YK11 ($p < 0.0001$), while in EXE had a decrease compared to EXE+YK11 ($p = 0.0471$); EXE+YK11 has an increase compared to control ($p = 0.0089$).

3.3. Antioxidant enzyme activity

SOD activity (Fig. 4A) was decreased in the YK11 group compared to control ($p < 0.0001$), EXE ($p < 0.0001$) and EXE+YK11 ($p = 0.0311$), while in EXE had an increased activity than control ($p = 0.0015$) and EXE +YK11 ($p < 0.0001$); EXE+YK11 has a decrease compared to

control ($p < 0.0001$). CAT activity (Fig. 4B) was decreased in the YK11 group compared to control ($p < 0.0001$), EXE ($p < 0.0001$), while in EXE had an increased activity regarding the EXE+YK11 ($p = 0.0001$); EXE+YK11 has a decrease compared to control ($p < 0.0001$).

GSH levels (Fig. 4C) was decreased in YK11 compared to control ($p < 0.0001$), EXE ($p < 0.0001$), while in EXE had an increased regarding the control ($p = 0.0104$) and EXE+YK11 ($p < 0.0001$); EXE+YK11 has a decrease compared to control ($p = 0.0008$). GPx activity (Fig. 4D) was decreased in YK11 compared to control ($p = 0.0005$), EXE ($p < 0.0001$), while in EXE had an increased regarding the EXE+YK11 ($p < 0.0001$); EXE+YK11 has a decrease compared to control ($p = 0.0002$). No statistical difference was found in GR activity (Fig. 4E). GST activity (Fig. 4F) was decreased in YK11 compared to control ($p = 0.0047$), EXE ($p < 0.0001$), while in EXE had an increased regarding the EXE+YK11 ($p = 0.0002$); EXE+YK11 has a decrease compared to control ($p = 0.01$).

3.4. Antioxidant and proteotoxicity regulators

Representative immunoblotting of antioxidant and proteotoxic regulators (Fig. 5A). Western immunoblotting of Nrf2 (Fig. 5B) was decreased in the YK11 group compared to control ($p = 0.0077$), and EXE

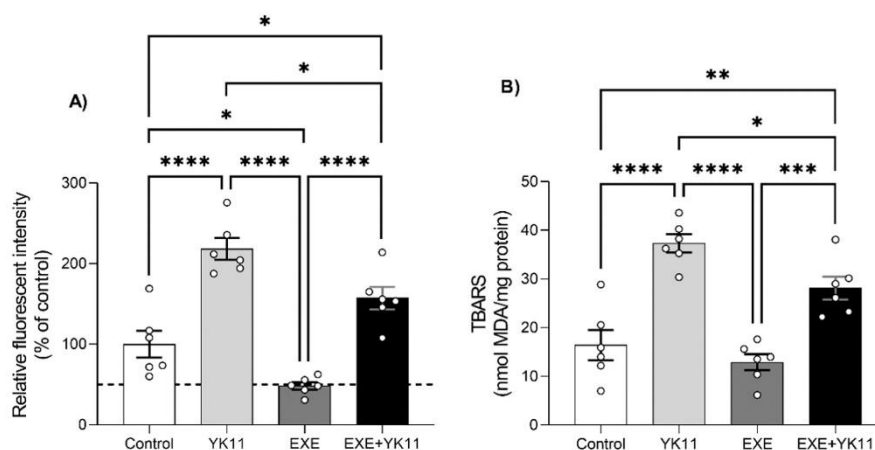


Fig. 3. Effects of YK11 administration (0.35 g/kg) and exercise (5-weeks) in oxidative stress markers indicated on A) RS levels; B) TBARS assay. Values are provided as mean and S.E.M (n = 6). Significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

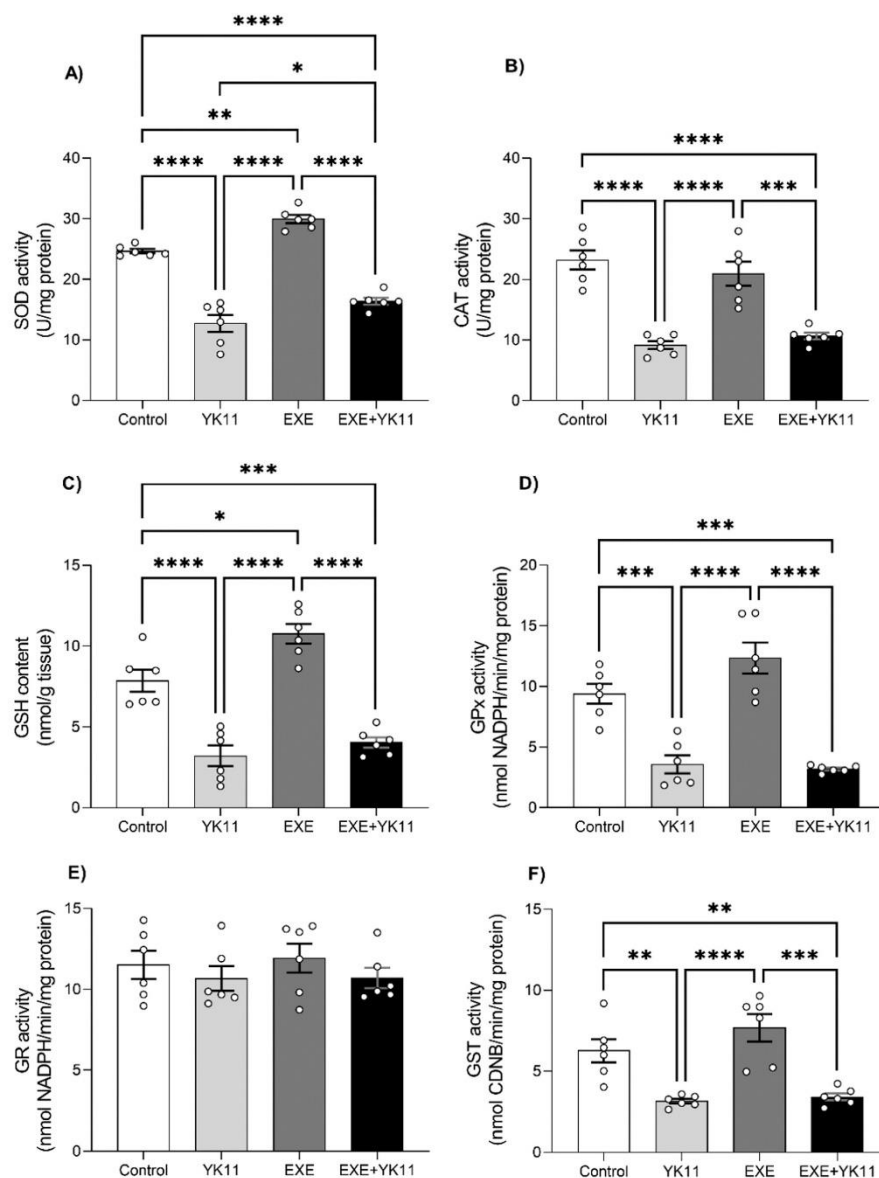


Fig. 4. Effects of YK11 administration (0.35 g/kg) and exercise (5-weeks) in antioxidant defense parameters indicated on A) SOD activity; B) CAT activity; C) GSH levels; D) GPx activity; E) GR activity; F) GST activity. Values are provided as mean and S.E.M (n = 6). Significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

($p < 0.0001$), while EXE had an increased regarding the control ($p = 0.0011$), and EXE+YK11 ($p < 0.0001$); EXE+YK11 has a decrease compared to control ($p = 0.0054$). Western immunoblotting of Hsp-70 (Fig. 5C) was increased in the YK11 group compared to control ($p = 0.0002$), EXE ($p = 0.0042$), and EXE+YK11 ($p = 0.0012$).

3.5. Mitochondrial toxicity markers

Representative immunoblotting of mitochondrial toxicity marker MnSOD/SOD2 (Fig. 6A). Western immunoblotting of MnSOD/SOD2 (Fig. 6B) was decreased in the YK11 group compared to control ($p = 0.0314$), and EXE ($p < 0.0001$), while EXE had an increased

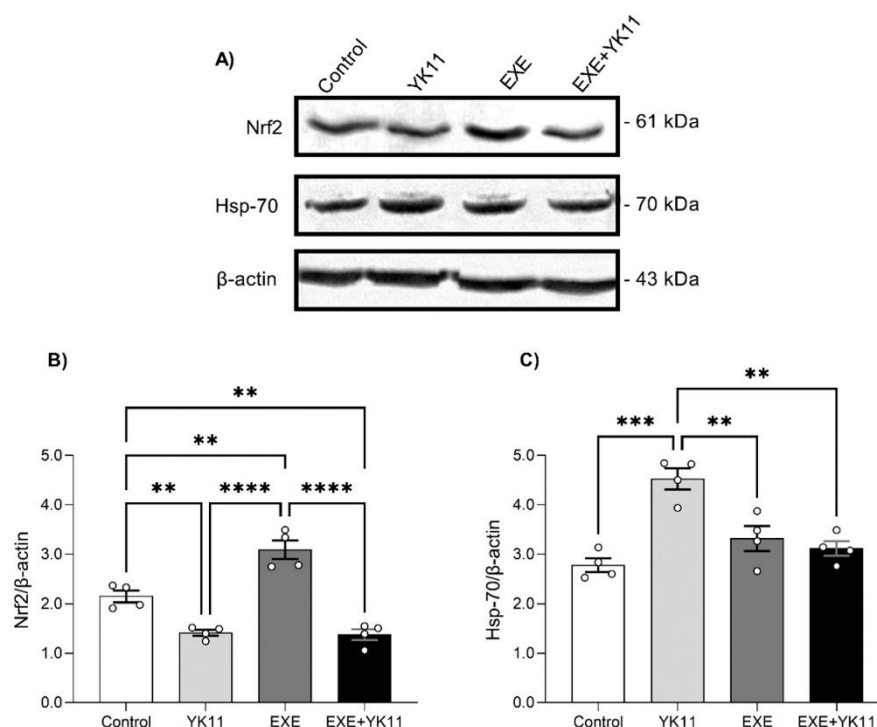


Fig. 5. Effects of YK11 administration (0.35 g/kg) and exercise (5-weeks) in oxidative stress and proteotoxic immunoblotting parameters indicated on A) Representative immunoblotting of antioxidant and proteotoxic markers; B) Nrf2 immunoreactivity; C) Hsp-70 immunoreactivity. β -actin represent as loading control. Values are provided as mean and S.E.M (n = 4). Significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

regarding the control ($p = 0.0173$), and EXE+YK11 ($p = 0.0038$). Mitochondrial viability (Fig. 6C) was decreased in the YK11 group compared to the control ($p = 0.0025$), EXE ($p < 0.0001$) and EXE+YK11 ($p = 0.0378$), while in EXE had an increase regarding to control ($p = 0.0055$), and EXE+YK11 ($p = 0.0003$). Of note, YK11 showed an average of $\approx 43\%$ of MTT reduction (below 50% capacity).

3.6. Mitochondrial respiratory chain complexes markers

Representative immunoblotting of regulatory proteins for mitochondrial respiratory chain complexes I, III and V (Fig. 7A). Western immunoblotting of CYTB (complex I) (Fig. 7B) was decreased in the YK11 group compared to control ($p = 0.0007$), and EXE ($p = 0.0004$), while EXE had an increase regarding the EXE+YK11 ($p < 0.0001$); EXE+YK11 has a decrease compared to control ($p < 0.0001$). Western immunoblotting of NDUFBS (complex III) (Fig. 7C) was decreased in the YK11 group compared to control ($p = 0.0023$), and EXE ($p = 0.0004$), while EXE had an increased regarding the EXE+YK11 ($p = 0.0010$); EXE+YK11 has a decrease compared to control ($p = 0.0065$). Western immunoblotting of ATP5A1 (complex V) (Fig. 7D) was decreased in the YK11 group compared to control ($p = 0.0219$), EXE ($p < 0.0001$), while EXE had an increased regarding the control ($p = 0.0008$), and EXE+YK11 ($p < 0.0001$); EXE+YK11 has a decrease compared to control ($p = 0.0276$).

3.7. Mitochondrial dynamic markers

Representative immunoblotting of mitochondrial dynamics biomarkers (Fig. 8A). Western immunoblotting of Mfn1 (Fig. 8B) was decreased in the YK11 group compared to control ($p < 0.0001$), and EXE ($p < 0.0001$), while EXE had an increased regarding the control ($p = 0.0004$), and EXE+YK11 ($p < 0.0001$). Western immunoblotting of Mfn2 (Fig. 8C) was decreased in the YK11 group compared to control ($p = 0.0147$), and EXE ($p < 0.0001$), while EXE had an increased regarding the control ($p = 0.0031$), and EXE+YK11 ($p < 0.0001$). Western immunoblotting of Drp1^{S637} (Fig. 8D) was increased in the YK11 group compared to control ($p = 0.0003$), EXE ($p < 0.0001$), while EXE had a decreased regarding the EXE+YK11 ($p = 0.0007$). EXE+YK11 has an increase compared to control ($p = 0.0085$).

4. Discussion

Our study aimed to investigate the neurochemical effects of co-administering YK11, a potent anabolic substance, and EXE, a major neuroprotective factor, in the hippocampus of male rats. Based on its molecular similarity to AAS, our main hypothesis was that YK11 increases cellular redox imbalance, resulting in elevated oxidative stress and affecting mitochondrial metabolism. In contrast, EXE was expected to mitigate these effects. Our findings revealed that YK11 disrupts redox balance and its associated regulatory proteins, leading to mitochondrial dysfunction and affecting the regulatory response of mitochondrial

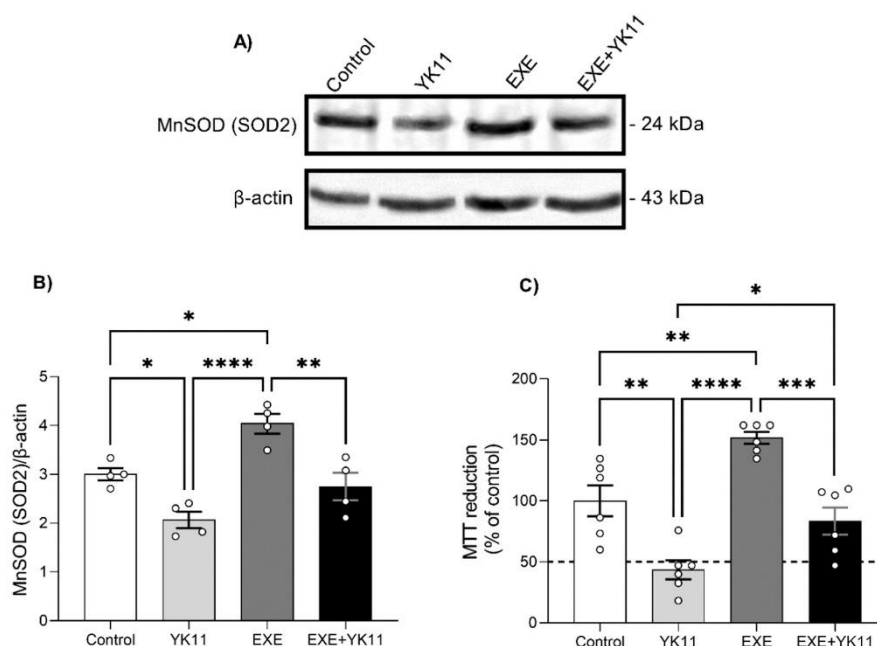


Fig. 6. Effects of YK11 administration (0.35 g/kg) and exercise (5-weeks) in mitochondrial cytotoxicity markers and immunoblotting parameters indicated on A) Representative immunoblotting of mitochondrial cytotoxicity markers; B) MnSOD/SOD2 immunoreactivity; C) MTT reduction assay. β -actin represent as loading control. Values are provided as mean and S.E.M (n = 4–6). Significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

respiratory chain subunits and mitochondrial fission and fusion proteins. Notably, these effects were observed regardless of EXE co-administration. In our work, initially we will discuss the metabolism of YK11, including its interaction with AR, and its primary effects on the hippocampus, given the high neurotoxicity of AAS in this tissue [4,6,8]. After, we elucidated the regulatory mechanisms of the YK11 and EXE co-administration, an increasingly prevalent anabolic combination worldwide [16,17].

The increasing use of SARMs, purported as having effects similar to AAS but without the associated side effects caused by these substances [3], raises concerns, particularly within the fitness community, where dosages and usage durations often exceed those indicated in clinical studies [14,36]. Notably, studies conducted by Vignali et al. [14] and Burmeister et al. [36] revealed a remarkable surge in SARM usage between 2020 and 2022, accompanied by a tendency to surpass clinically tested dosages. Considering these trends, our study aims to provide valuable insights into crucial issues surrounding the worldwide use of SARMs. We specifically focus on YK11, which is claimed to be a mighty myostatin inhibitor [10], a characteristic that we believe contributes to its increased potential for misuse. By delving deeper into the topic of high SARM dosages, we address a crucial and influential factor among users of these substances, offering new perspectives and a better understanding of this evolving field.

Considered the most potent SARM on the market [16,17], the anabolic effect of YK11 is mainly due to its interaction with AR, through the increase in FST levels, causing a facilitatory effect on myostatin inhibition, the main regulator of protein synthesis and muscle growth, modulating the mammalian target of rapamycin (mTOR) [16]. Compared to other SARMs, YK11 exhibits a higher level of similarity to AAS derivatives, particularly those derived from testosterone, due to its

possession of four steroid rings [37]. This unique chemical configuration enables YK11 to engage in molecular interactions similar to AAS, while still maintaining the specific androgen receptor interaction characteristic of a SARM [38]. However, it is crucial to note that the presence of this configuration also implies a greater potential for androgenic side effects, including hormone suppression, mood alterations, and particularly neurochemical impairments [38], which may not be observed in other SARMs lacking this steroid configuration, such as ligandrol and ostarine [37].

Nevertheless, our understanding of YK11 metabolism with AR remains limited. Thus, we conducted molecular docking comparisons between YK11 and AR, employing DHT (the native ligand for AR) as a control model. The results from our molecular docking analysis demonstrate that YK11 exhibits a predicted ΔG_{bind} affinity with AR similar to DHT. Notably, both compounds share interactions with Arg752, a crucial binding site known for its role in the activation of steroid ligands. Mutations in this site have been shown to compromise the affinity of interaction with AR, affecting their activation. Moreover, the molecular characteristics of YK11, featuring four aromatic structures resembling testosterone and its derivatives, suggest a potential for increased side effects and a similar metabolism pathway [38]. It is worth mentioning that these compounds are commonly metabolized by cytochrome P450 enzymes (CYP450) [39], which can lead to the generation of higher levels of free radicals through intensified oxidative reactions [40]. Based on these observations, we propose a hypothesis that YK11 may induce similar effects, triggering a cascade effect caused by elevated levels of free radicals within the CNS, particularly in the hippocampus.

The continuous increase in the metabolism of steroid derivatives can lead to the formation of free radicals within neuronal cells [41,42]. In

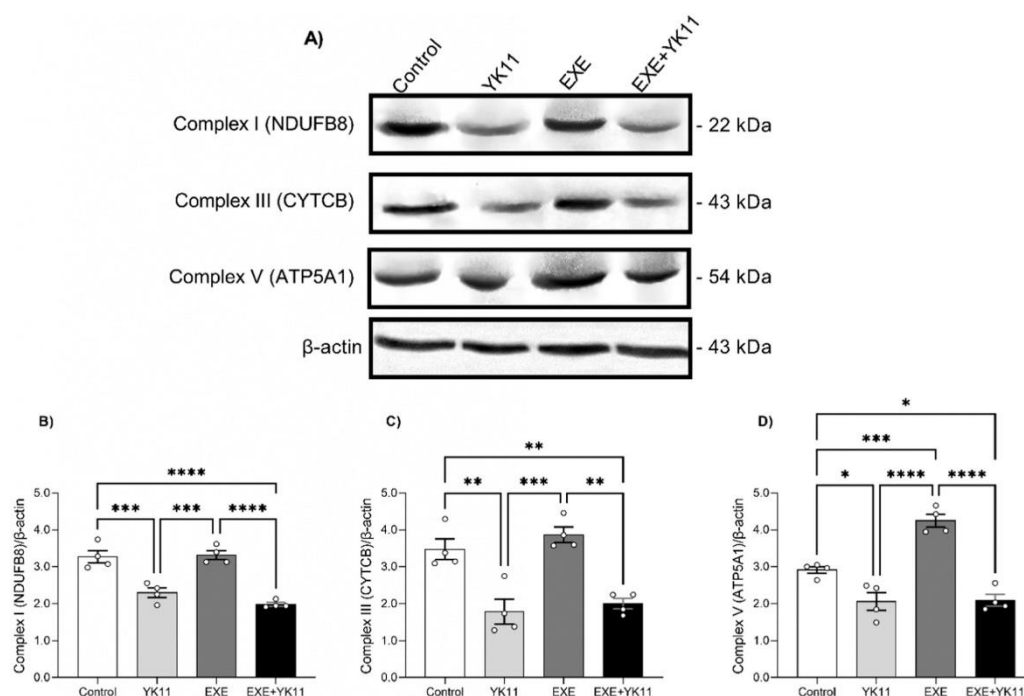


Fig. 7. Effects of YK11 administration (0.35 g/kg) and exercise (5-weeks) in mitochondrial respiratory chain complexes immunoblotting parameters indicated on A) Representative immunoblotting of mitochondrial respiratory chain complexes markers; B) Complex I (NDUFB8) immunoreactivity; C) Complex III (CYTCB) immunoreactivity; D) Complex V (ATP5A1) immunoreactivity. β -actin represent as loading control. Values are provided as mean and S.E.M (n = 4). Significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

our study, we found that YK11 increases RS formation in the rat hippocampus, associated with an elevation in MDA levels, a lipid peroxidation biomarker. Furthermore, YK11 caused changes in the endogenous antioxidant system by altering the activity of several regulators involved in antioxidant and proteotoxic pathways. Specifically, YK11 reduced SOD/CAT activity, Nrf2/glutathione system, and Hsp-70 levels. Our hypothesis is that organic peroxides generated in response to YK11 positively regulate Hsp-70, a proteotoxic inducer, which limits the release of Nrf2 from the Kelch-like ECH-associated protein 1 subunit (Keap-1), impairing its translocation to the nucleus. The modulation of the Nrf2/antioxidant-responsive element (ARE) system in DNA promotes changes in SOD, leading to increased O_2 levels in the cell and inactivation of CAT [42,43]. In parallel, YK11 produces changes in the regulation of glutathione-mediated antioxidant defense, promoting a decrease in GPx and GST activity, and as expected, causing a reduction in GSH levels, the main reducing substrate in organic peroxide transformation [44]. Notably, our results indicate that the neurochemical impairments caused by YK11 do not involve alterations in GR activity. These effects are similar to those observed in the rat hippocampus that were administered with several AAS, which promote an increase in free radical production associated with a decreased ratio in antioxidant activity/enzyme antioxidant renewal cycle [44–46].

The increased oxidative stress caused by YK11 may result in cellular energetic decline, leading to mitochondrial dysfunction and activation of the intrinsic pathway metabolism of pro-apoptotic inducers, such as caspase 3/7 [6,7]. Therefore, we confirmed that YK11 reduces the activity of MnSOD/SOD2, an enzyme present in the mitochondria that converts $O_2^{\cdot -}$ into H_2O_2 through a mechanism of restricted diffusion,

thereby limiting the amount of radicals released into the cell [48]. Furthermore, we observed a decreased capacity for MTT reduction in the hippocampus of rats administered with YK11, indicating reduced mitochondrial activity and fewer metabolically viable cells [49]. The increased mitochondrial toxicity observed suggests a possible dysregulation of the main complexes of the mitochondrial respiratory chain [50]. Our findings demonstrate that YK11 suppresses the activity of mitochondrial chain complexes I, III, and V in the hippocampus, limiting energy production in the cell, and possibly indicating the main neurodegenerative mechanism associated with YK11. Decreased mitochondrial oxidative phosphorylation proteins, such as those found in complexes I, III, and IV, can lead to lower metabolic efficiency, which ultimately affects the transfer of electrons from NADH to ubiquinone and cytochrome c [50]. In turn, this results in decreased ATP synthesis, which can significantly impact cellular processes, including defects in mitophagy and mitochondrial dynamics such as fission and fusion [51, 52].

Several studies have demonstrated the importance of mitochondrial dynamics and the regulation of mitochondrial fission and fusion proteins in the proper functioning of the hippocampus [51–54]. Our results demonstrate that YK11 alters mitochondrial fusion markers by decreasing Mfn1 and Mfn2 levels, which are essential for tethering adjacent mitochondria and facilitating their interactions with GTPases and other proteins involved in the mitochondrial fusion process [52]. Additionally, YK11 increases the levels of Drp1^{S637}, which promotes mitochondrial fission [53]. This result is mainly associated with the reduction of outer mitochondrial membrane (OMM) proteins (Mfn1/2) that regulate the recruitment of Drp1^{S637} from the cytoplasm to

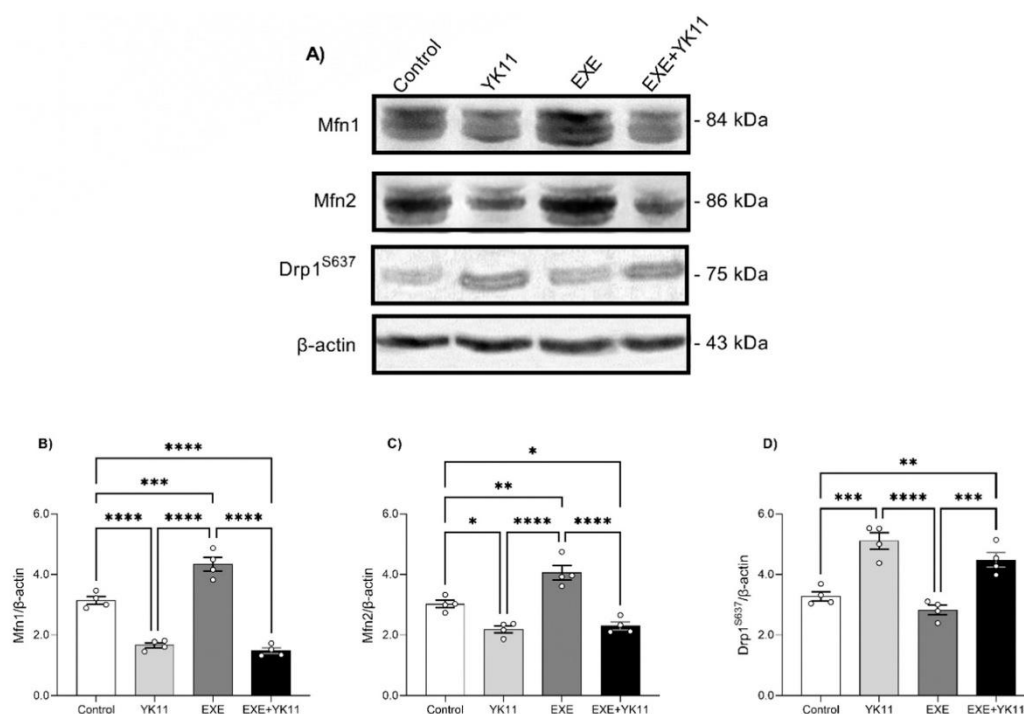


Fig. 8. Effects of YK11 administration (0.35 g/kg) and exercise (5-weeks) in mitochondrial dynamic immunoblotting parameters indicated on A) Representative immunoblotting of mitochondrial dynamic markers; B) Mfn1 immunoreactivity; C) Mfn2 immunoreactivity; D) Drp1^{S637} immunoreactivity. β-actin represent as loading control. Values are provided as mean and S.E.M (n = 4). Significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

mitochondria [53]. Therefore, the proper functioning of mitochondrial fusion and fission processes is dependent on the regulation of these proteins, and any alterations in their expression can lead to general failures in mitochondrial dynamics mechanisms [51–53].

In contrast, the EXE group appears to exhibit antioxidant effects in the hippocampus of rats, such as found in several studies using EXE in rodents [41,42]. However, the co-administration of EXE+YK11 does not prevent the pro-oxidant effect of YK11. Some studies support the idea that AAS hinder the neuroprotective effect of EXE by altering apoptotic signaling pathways (Bax/Bcl-2) and neuroplasticity mechanisms (Brain Derived Neurotrophic Factor [BDNF]), which are linked to antioxidant mechanisms [46,55]. However, other studies present conflicting results regarding the pro-oxidant effects of AAS, which seem to be reversed by EXE, leading to the restoration of SOD activity and a reduction in MDA levels, indicating the maintenance of redox balance in the hippocampus [55,56]. Despite the controversy, these divergent results are derived from several factors, including the type of exercise induced and the dose of AAS. Therefore, our findings are crucial due to the limited number of studies that explore the action of SARMs in modulating the antioxidant system in the hippocampus, providing insight into some of the mechanisms involved.

The EXE protocol has been shown to be an important regulator of energy metabolism, as it increases the levels of MnSOD/SOD2 and the number of metabolically viable cells. Similarly, a study by Lin et al. [56] demonstrated that EXE induction up-regulates SOD2, reducing the activation of intrinsic mitochondrial-dependent apoptotic pathways. Additionally, Cechetti et al. [47] found a correlation between EXE and

mitochondrial biogenesis, as well as increased metabolic viability in the hippocampus. Our findings suggest that the combination of EXE+YK11 can prevent the decrease in certain mitochondrial toxicity markers by restoring MnSOD/SOD2 levels and maintaining metabolically viable cells. These results support the study conducted by Gomes et al. [46], which demonstrated that the combination of EXE with AAS can prevent a decline in SOD activity and restore energy metabolism in hippocampal cells. However, further investigation is required to explore the effects of this combination on intermediate mechanisms of mitochondrial regulation, such as the activity of mitochondrial complexes and regulatory proteins involved in mitochondrial dynamics.

Mitochondrial complexes play a crucial role in regulating cellular energy metabolism, and their maintenance is essential for cellular health [54]. Our results demonstrate that EXE increases the activity of complex V, also known as ATP synthase, the key enzyme responsible for ATP synthesis, the primary source of cellular energy [57]. This observation is in line with the study by Gusdon et al. [58], who showed that EXE regulates the activity of mitochondrial respiratory chain complexes in structures such as the cortex, striatum, and cerebellum of rats. Our main hypothesis is that EXE promotes the regulation of several mechanisms associated with neuromodulation, including the signaling of neurotrophic factors, increasing neuroplasticity [59], in addition to supporting vital cellular processes, such as maintaining redox potential, regulating apoptotic pathways, and ensuring intracellular calcium (Ca²⁺) homeostasis, mechanisms that require high energy demands for the brain [60]. In contrast, EXE+YK11 fails to prevent the decreased activity of mitochondrial complexes I, III, and V, suggesting that EXE

may not be able to counteract YK11 impairments to the mitochondrial respiratory chain. However, despite the impairments in complex V, EXE+YK11 prevents changes in the metabolic efficiency of cells, preserving overall mitochondrial dehydrogenase metabolism, indicating some level of protection in the co-administration of EXE+YK11, particularly indicating a metabolic fraction that is not altered by YK11.

Additionally, we observed that EXE regulates mitochondrial dynamics by increasing the levels of Mfn1/Mfn2 and decreasing the levels of Drp1^{S637}, thereby promoting mitochondrial fusion, and inhibiting mitochondrial fission. These findings are consistent with the study by Yan et al. [53], which showed that treadmill exercise regulates mitochondrial dynamics markers in the hippocampus, acting as a protective factor in the development of neurodegenerative diseases. In contrast, the combination of EXE+YK11 decreases the levels of Mfn1/Mfn2 and increases the levels of Drp1^{S637}, indicating significant changes in mitochondrial metabolism. Since mitochondrial fusion and fission proteins are interdependent [53,58], alterations in their expression can extensively disrupt mitochondrial dynamics mechanisms.

5. Conclusion

Collectively, we found that high doses of YK11, a potent SARM purposed as a safer alternative to AAS, exhibits neurochemical impairments in the hippocampus similar to those substances, regardless of EXE co-administration. YK11 increases oxidative stress and disrupts proteotoxic markers associated with mitochondrial dysfunction. Although EXE shows its expected neuroprotective effect by enhancing antioxidant defenses and regulating mitochondrial function, the combination of EXE+YK11 does not protect against these neurochemical impairments caused by YK11. Notably, the protective effect of EXE+YK11 is limited to maintaining MnSOD/SOD2 levels and preserving mitochondrial dehydrogenases viability. In this sense, comprehending the potential risks and implications of using high doses of YK11 is crucial for making informed decisions about its administration. The increasing misuse of SARMS is driven by the perception of obtaining AAS-like effects with minimal adverse reactions. However, additional studies are necessary to investigate the effects at lower doses and in combination with exercise.

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CRediT authorship contribution statement

Mustafa Munir Mustafa Dahleh: Conceptualization, Data curation, Formal analysis, Investigation, Software, Methodology, Supervision, Validation, Visualization, Roles/Writing – original draft, Writing – review & editing. **Vandrezza Cardoso Bortolotto:** Investigation, Methodology, Software, Supervision, Validation, Visualization. **Gustavo Petri Guerra:** Data curation, Formal analysis, Funding acquisition, Investigation, Writing – review & editing. **Silvana Peterini Boeira:** Funding acquisition, Supervision. **Marina Prigol:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Software, Methodology, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing for financial interests or personal relationships.

Data Availability

Data will be made available on request.

Appendix A. Supporting information

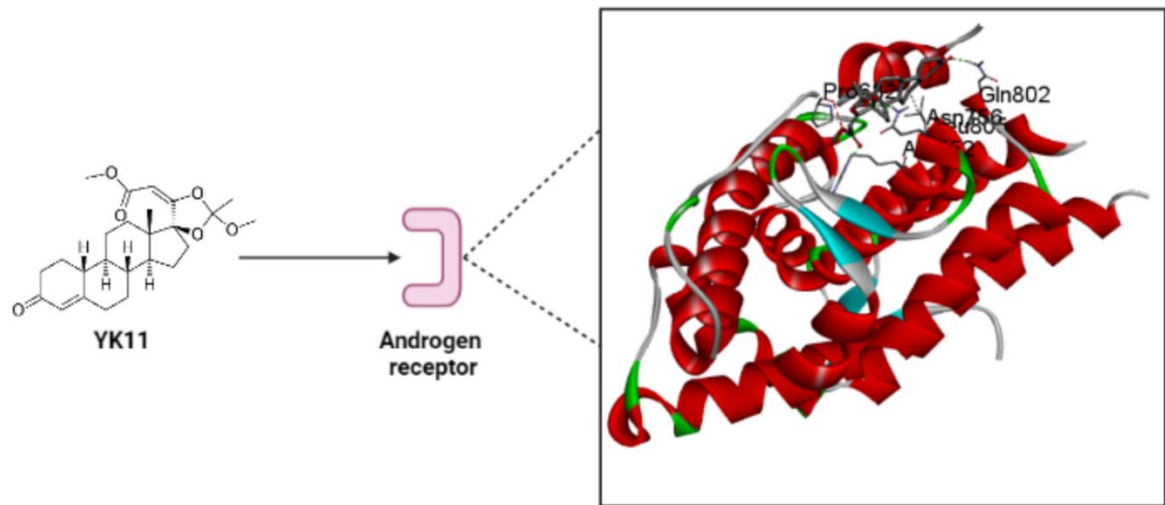
Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jsbmb.2023.106364.

References

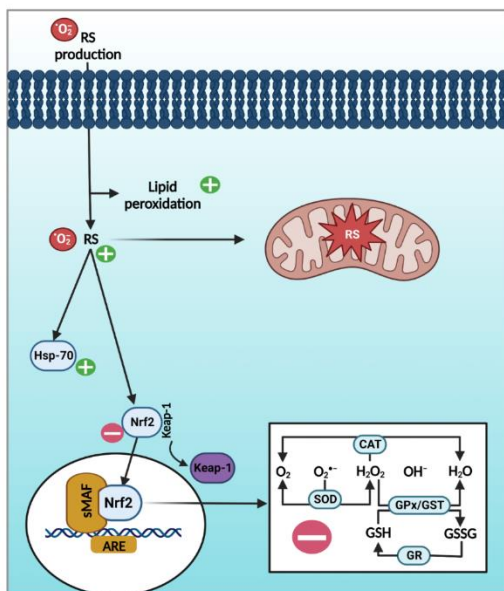
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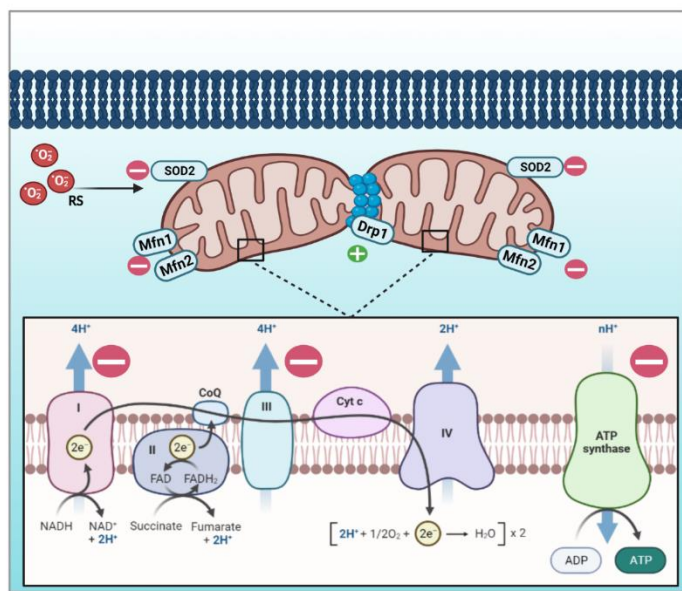
Graphical Abstract – Artigo científico 1



Oxidative stress signaling of YK11 in hippocampus



Mitochondrial signaling of YK11 in hippocampus



5.1.2. Material suplementar: Artígo científico 1

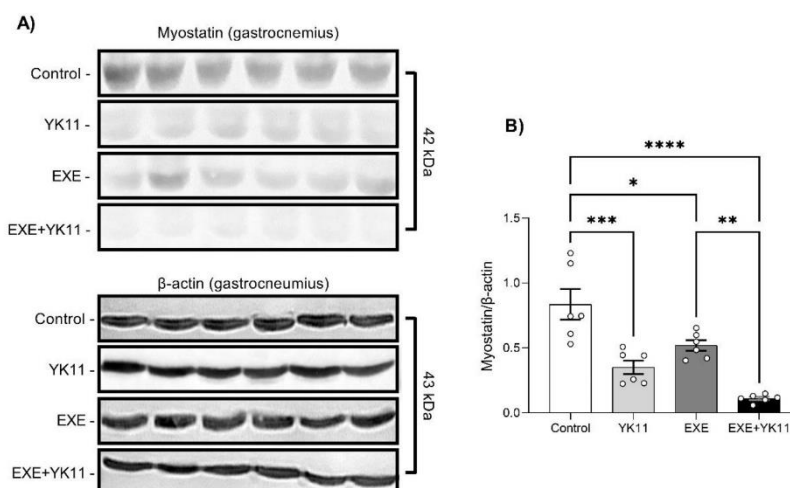


Figure S1. Effects of YK11 administration (0.35 g/kg) and exercise (5-weeks) on myostatin expression in rat gastrocnemius confirming myostatin inhibitory potential by YK11 and EXE. The same animals tested (n = 6) for muscle myostatin immunoexpression were used to determine the neurochemical parameters. A) Representative immunoblotting of myostatin and β -actin as loading control; B) Myostatin/ β -actin ratio. Values are provided as mean and S.E.M (n = 6). Significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

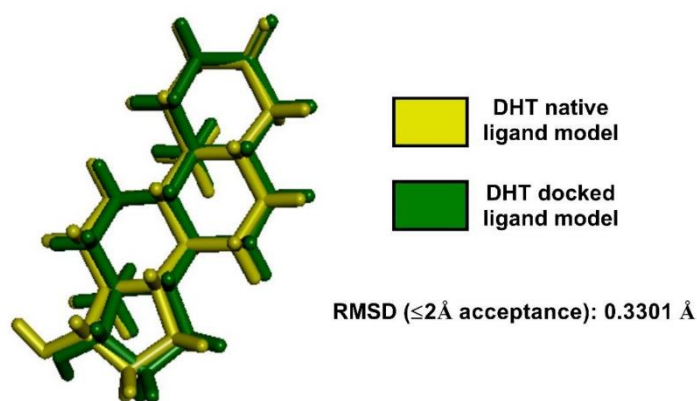


Figure S2. Molecular docking validation was performed in DHT native ligand by redocking DHT ligand with AR. DHT native ligand are represented in yellow, and DHT

redocked ligand in green. The validation model between DHT native ligand and DHT redocked ligand shows a strong RMSD = 0.3301 Å (≤ 2 Å acceptance).

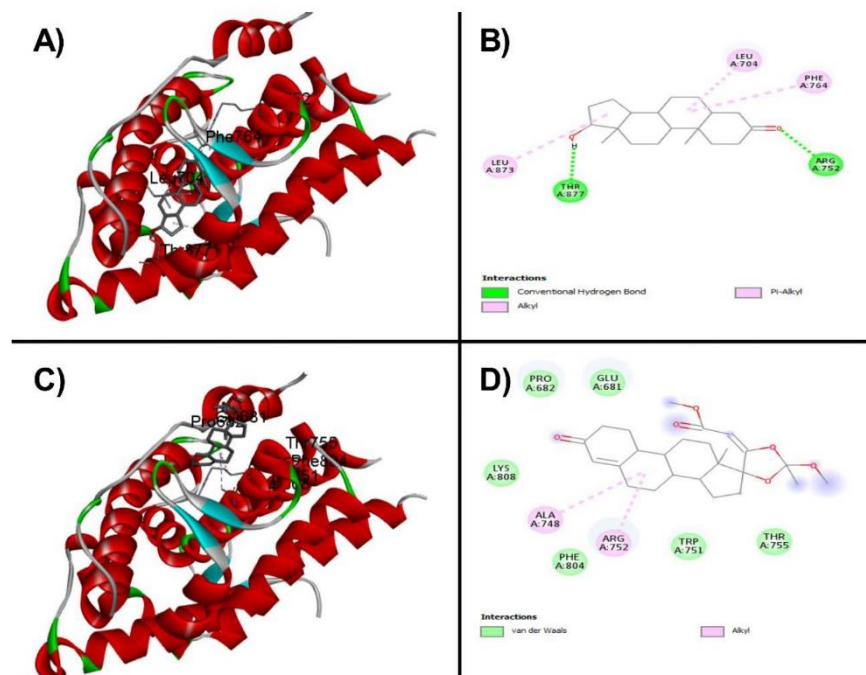


Figure S3. Protein-ligand interactions between native DHT and YK11 species. A and B) 3D and 2D representation between AR-DHT interactions; C-D) 3D and 2D representation between AR-YK11 interactions. Data obtained using the software AutoDockVina and Discovery Studio Visualizer.

5.2. Artigo científico 2

ARTIGO: From gains to gaps? How Selective Androgen Receptor Modulator (SARM) YK11 impact hippocampal function: *In silico*, *in vivo*, and *ex vivo* perspectives

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Research paper

From gains to gaps? How Selective Androgen Receptor Modulator (SARM) YK11 impact hippocampal function: *In silico*, *in vivo*, and *ex vivo* perspectives

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ABSTRACT

Selective Androgen Receptor Modulators (SARMs), particularly (17 α ,20E)-17,20-[(1-methoxyethylidene)bis(oxy)]-3-oxo-19-norpregna-4,20-diene-21-carboxylic acid-methyl-ester (YK11), are increasingly popular among athletes seeking enhanced performance. Serving as an Androgen Receptor (AR) agonist, YK11 stimulates muscle growth while inhibiting myostatin. Our study delved into the impact of YK11 on the rat hippocampus, analyzing potential alterations in neurochemical mechanisms and investigating its synergistic effects with exercise (EXE), based on the strong relationship between SARM users and regular exercise. Utilizing Physiologically Based Pharmacokinetic (PBPK) modeling, we demonstrated YK11 remarkable brain permeability, with molecular docking analysis revealing YK11 inhibitory effects on 5- α -reductase type II (5 α R2), suggesting high cell bioavailability. Throughout a 5-week experiment, we divided the animals into the following groups: Control, YK11 (0.35 g/kg), EXE (swimming exercise), and EXE + YK11. Our findings showed that YK11 displayed a high binding affinity with AR in the hippocampus, influencing neurochemical function and modulating aversive memory consolidation, including the downregulation of the BDNF/TrkB/CREB signaling, irrespective of EXE combination. In the hippocampus, YK11 increased pro-inflammatory IL-1 β and IL-6 cytokines, while reducing anti-inflammatory IL-10 levels. However, the EXE + YK11 group counteracted IL-6 effects and elevated IL-10. Analysis of apoptotic proteins revealed heightened p38 MAPK activity in response to YK11-induced inflammation, initiating the apoptotic cascade involving Bax/Bcl-2/cleaved caspase-3. Notably, the EXE + YK11 group mitigated alterations in Bcl-2 and cleaved caspase-3 proteins. In conclusion, our findings suggest that YK11, at anabolic doses, significantly alters hippocampal neurochemistry, leading to impairments in memory consolidation. This underscores concerns about the misuse risks of SARMs among athletes and challenges common perceptions of their minimal side effects.

1. Introduction

Selective Androgen Receptor Modulators (SARMs) have attracted significant attention in the realm of performance enhancement and muscle development [1]. These compounds, first identified during drug development trials in the early 1990s, are viewed as a potentially safer and less harmful alternative to anabolic androgenic steroids (AAS) [2]. The perceived safety of SARMs primarily stems from their selective action, which targets specific parts of the body, like striated muscles [1,3]

in contrast to AAS (Arnés and Casares, 2022), which exert widespread effects on various tissues, including the central nervous system (CNS) [4, 5] and the reproductive system [6].

A compelling example is (17 α ,20E)-17,20-[(1-methoxyethylidene) bis(oxy)]-3-oxo-19-norpregna-4,20-diene-21-carboxylic acid methyl ester, commonly referred as YK11 [7]. This distinctive compound operates in a manner akin to a SARM and is classified as part of that class, despite possessing the chemical structure and backbone of an AAS [7,8]. YK11, acclaimed for its capacity to stimulate rapid muscle growth,

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is characterized as a steroid SARM, functioning as a partial Androgen Receptor (AR) agonist [9,10]. Also, it is known to act as an indirect inhibitor of myostatin, a protein that limits muscle mass fast development, thereby fostering a significant enhancement in the hypertrophy and hyperplasia of muscle microfibrils through positive signaling in the synthesis of the follistatin protein [9,11], particularly when coupled with regular exercise [11]. For these reasons, YK11 is often considered the most potent of the SARMS [10,12].

The misuse of these substances, particularly by athletes aiming for performance enhancement, is a significant concern. The intake of high doses, often classified as anabolic, can lead to severe side effects and present considerable health risks [13,14]. While AAS adverse effects are well-documented, the full scope of SARMS effects remains unclear. This knowledge gap is troubling, given the substantial increase in demand, reaching 115 million by 2021 [15]. Significantly, the trend in SARMS usage mirrors that of AAS, with an annual increase of approximately 3.3% [14], exhibiting a higher prevalence among men than women, with a ratio of 2.6:1 concerning the use of ergogenic substances [16]. This escalation in usage raises concerns, particularly among male users, highlighting the pressing need to address the implications of these substances. Despite their appeal for performance enhancement, SARMS are banned by the World Anti-Doping Agency (WADA/USADA) due to their ergogenic potential [17]. Although the Food and Drug Administration (FDA) has not approved SARMS for human use, athletes easily access and often dangerously consume these substances [18].

In this sense, it is crucial to comprehend the potential risks tied to such usage patterns to establish safe practices and regulatory measures, with a specific emphasis on their neurotoxic effects, as observed in AAS side effects [19,20]. In this context, Dahleh et al. [11] have shown that YK11, when administered at high anabolic doses, can escalate oxidative stress, and induce dysfunction in mitochondrial metabolism within the hippocampus. Despite the evident adverse impact on the neurochemistry of the rat hippocampus, a substantial suppression of myostatin was observed in the gastrocnemius, particularly when YK11 was combined with regular exercise. This highlights a clear relation and involvement between anabolic dose and toxic effects, a practice commonly undertaken by both amateur and professional athletes. Of note, this happens irrespective of simultaneous engagement in concurrent exercise, a factor broadly acknowledged as a neuroprotective agent [21,22].

The emergence of SARMS, particularly YK11, has ushered in new possibilities for muscle growth and fat loss, especially when incorporated into regular exercise regimes [1]. However, the dearth of comprehensive research into its metabolism and potential side effects, including neurotoxicity, necessitates a prudent approach to its usage. To address these concerns, we employed a systematic approach utilizing *in silico*, *in vivo*, and *ex vivo* models. This comprehensive strategy was chosen as data obtained from these three models converge to yield consistent results regarding the primary effects of YK11 on the CNS. Physiologically Based Pharmacokinetic (PBPK) modeling was chosen in our study for its capacity to predict the remarkable brain permeability of YK11, shedding light on potential interactions between drugs and the CNS [23]. Also, molecular docking and dynamics were utilized to explore YK11 inhibitory effects on 5-alpha-reductase type II (5αR2), providing insights into its cellular bioavailability [24], as well as its investigation into the affinity for AR in the hippocampus, along with its impact on memory mechanisms and related behaviors, inflammation, and apoptosis, remarkable effects on CNS by AAS abuse [19,25]. This broadened our understanding of YK11 potential selectivity for the hippocampal AR and its implications for tissue metabolism. In this context, our aim was to clarify the potential neurotoxic mechanisms linked to the metabolism of YK11, to understand how the presence of this compound in the CNS can influence and modify the intricate neurochemical mechanisms inherent to the hippocampus.

2. Material and methods

2.1. Chemicals

3,3',5,5'-Tetramethylbenzidine (TMB), aprotinin, β-glycerolphosphate, bromophenol blue, dithiothreitol (DTT), ethylenediaminetetraacetic acid (EDTA), glycerol, HEPES, magnesium chloride (MgCl₂), phenylmethylsulfonyl fluoride (PMSF), potassium chloride (KCl), sodium chloride (NaCl), sodium fluoride (NaF), and sodium orthovanadate were acquired from Sigma-Aldrich® (St. Louis, MO, USA). YK11, along with other compounds, was sourced either in analytical grade or from established commercial providers.

2.2. Animals

Male Wistar rats (200–250 g; 60-day-old), obtained from the Central Biotery of the Federal University of Santa Maria (Rio Grande do Sul, Brazil), were utilized for the study. A total of twenty-four rats were employed in the experimental layout. These animals were kept within acrylic cages, placed in a temperature-controlled environment (22 ± 2 °C), and subjected to a 12:12 h light/dark cycle (lights on 7:00 a.m., off 7:00 p.m.). Adequate nourishment and water were made available *ad libitum*. The methodology for all investigations detailed in this research adhered to the regulatory guidelines established by the Brazilian Council of Animal Experimentation (CONCEA), as well as the U.S. Public Health Service's Policy on Humane Care and Use of Laboratory Animals (PHS Policy), and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). The study protocol was reviewed and sanctioned by the local Ethics Committee for Animal Research of the Federal University of Pampa (CEUA protocol 009/2022).

2.3. Experimental protocol

Sixty-days-old male Wistar rats were randomly divided into four distinct groups: Control (Vehicle, no YK11, no exercise), YK11 (Vehicle, YK11 0.35 g/kg, no exercise), EXE (Vehicle, no YK1, exercised), EXE + YK11 (Vehicle, YK11 0.35 g/kg, exercised). The vehicle solution (1 mL/kg of 1% ethanol dissolved in 0.9% saline) or YK11 (0.35 g/kg in 1% ethanol dissolved in 0.9% saline) was administered orally once daily over a five-week period (Fig. 1). The selection of male Wistar rats for our experiments is attributed by the significantly higher prevalence of ergogenic substance abuse observed in men compared to women, with a ratio of 2.6:1 [16]. The chosen dosage of YK11 (0.35 g/kg/day) is the most recorded dosage of this SARM in rodent studies [10,11] and was selected to mimic a misuse scenario, reflecting the growing global trend of SARMS abuse. All animals used in the study were tested for myostatin immunoreactivity in the gastrocnemius muscle to confirm the anabolic effect of YK11. These results are present in our previous work, in which YK11 and EXE + YK11 obtained a partial inhibition of myostatin [11]. The five-week swimming exercise protocol was adapted from a study by Stone et al. [26] and involved a progressive load protocol of 0–2% of the rat's body weight, water depth of 20–40 cm, and duration of 20–50 min, five days per week (Table 1). On the 45th day, one day after to the final day of the swimming protocol, to minimize variables related to physical fatigue, the animals underwent behavioral testing. On the 46th day, the animals were euthanized, and the hippocampus was promptly extracted for *ex vivo* analysis. Given the ability of SARMS to cross the blood-brain barrier and primarily act in the hippocampus, like AAS, this tissue was used to assess the neurological effects of YK11.

2.4. *In silico* analysis

2.4.1. Physiologically Based Pharmacokinetic (PBPK) modelling

Pharmacokinetic profiles for Wistar rats were simulated using PK-SIM® Open System Pharmacology (version 11.2.142, update 2) using

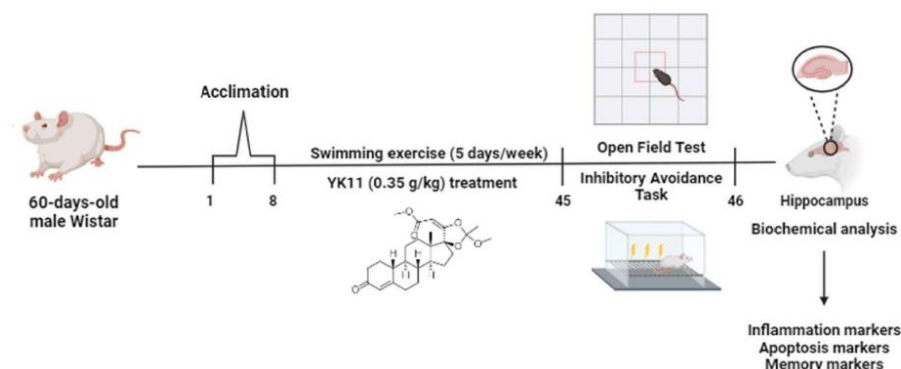


Fig. 1. Illustrative depiction of the experimental design.

Table 1
Five-week swimming training protocol schedule.

Week	Intensity (%) ^a	Water depth (cm)	Training (min/day)
1	0	20	20
2	0	30	30
3	1	30	30
4	1	30	40
5	2	40	50

^a Percentage of weight added in relation to the body weight of each rat.

default rats' metabolic model. The software used available data on SARMS pharmacokinetics and employed full PBPK distribution models to simulate the selected dose regimens, considering the mean weight of rats in the YK11 group. In line with the experimental design of a 5-week supplementation regimen, which included definition of oral administration route and a YK11 supplementation of 0.35 g/kg, the concentration of this SARM was examined over a 24 h metabolic period in various tissues - blood plasma, brain, striated muscle, and fat tissue. The PBPK basic modeling was conducted solely using predictive models, integrating the animals' body weight at the analyzed time points as inputs [27]. This approach ensures a more accurate and comprehensive understanding of the pharmacokinetic behavior of YK11 in the Wistar rats.

2.4.2. Molecular docking

Molecular docking analyses were conducted to assess the inhibitory potential of YK11 against type II 5 α -Reductase (5 α R2). The crystal structure of 5 α R2 was obtained from the Protein Data Bank (PDB) under ID code 7BW1 [28], and the crystal structure of YK11 was retrieved from the Cambridge Crystallographic Data Center (CCDC) under ID code 2178474 [29]. As comparative models for assessing YK11 potential to inhibit 5 α R2, the crystallographed finasteride (CCDC code 1294659, used as the positive control) (Wawrzycka et al., 1999) and crystallographed testosterone (PDB ID code 2AM9 ligand, serving as the negative control) [30] were docked to 5 α R2. To evaluate the accuracy of the docking protocol, redocking was performed using the native ligand (PDB ID code 7BW1) [28]. The docked positions were compared to the crystal structure positions by calculating root-mean-square deviation (RMSD) values. The native model was validated, demonstrating an RMSD of 0.3992 Å (within the acceptable range of ≤ 2 Å) [31]. In the docking simulations, the ligands were treated as flexible (PM6 charges), while the receptors were kept rigid (Gasteiger charges) using AutoDockTools [32]. Analysis of 5 α R2-YK11, 5 α R2-finasteride, and 5 α R2-testosterone interactions involved removing the native 7BW1 ligand, retaining 5 α R2 chain A free. For the docking simulations, AutoDockVina software [33]

was utilized. A grid box encompassed the entire 5 α R2 active site ($92 \times 71 \times 85$ Å) with coordinates $x = -32.264$, $y = 16.389$, $z = 37.36$, and an exhaustiveness setting of 100 was applied. The results were analyzed using AutoDockVina software and Discovery Studio Visualizer software.

2.4.3. Molecular dynamics

The GROMACS engine [34] were employed for all molecular dynamic simulations of ligand-protein complexes in explicit water. To evaluate the stability of these complexes, RMSD trajectory was calculated for each complex. Initial poses were derived from the 5 α R2-YK11, 5 α R2-finasteride, and 5 α R2-testosterone complexes, which were obtained through previous molecular docking.

For protein preparation, the AMBER99 forcefield was used [35], and the topology of the ligands was generated using the PRODRG tool [36]. The TIP3P water model was applied [37], and a triclinic water box was utilized for the protein-ligand complex. Prior to initiating the molecular dynamic simulations, a system minimization was performed with the steepest descent algorithm, consisting of 5000 steps, with the structures subject the NVT/NPT equilibrations. The simulations were conducted in the presence of 0.15 M NaCl ions under constant temperature conditions (298 K) and pressure (1.0 bar). Approximately 1000 frames were generated for each simulation, with each simulation running for 50 ns.

2.5. In vivo analysis

For *in vivo* analyses, all animals ($n = 5-6$) that were used to obtain confirmation of the androgenic potential of YK11 (YK11 or EXE + YK11) through myostatin immunoreaction analysis were subsequently used in behavioral analyses, as observed in our previous work [11]. This was done to effectively analyze the relationship between androgenic effects and behavioral modulation. Of note, it is important to note that the choice of a smaller sample size was guided by ethical considerations to minimize the use of animals in experiments based in our local Council of Animal Experimentation (CEUA protocol 009/2022).

2.5.1. Open Field Test (OFT)

Locomotor activity was assessed using the Open Field Test (OFT), where each animal was individually placed in a 45 cm² acrylic box with walls measuring 30 cm in height. Measurements including distance traveled (mm), the number of rearing, and velocity (mm/s) were recorded using the native equipment software during a 5 min session [38]. To prevent potential influences on the animals' movements, the apparatus was thoroughly cleaned with 70% alcohol between each trial, and a 3 min interval was allowed between procedures for each animal.

2.5.2. Inhibitory avoidance task

To assess memory consolidation, animals underwent a single training session in a step-down inhibitory avoidance apparatus. This apparatus consisted of a $25 \times 25 \times 35$ -cm box with a grid floor, and the left portion was covered by a 7×25 -cm platform, which was 2.5 cm in height. The rats were gently placed on the platform, facing the rear left corner. When the rats placed all four paws on the grid, a 3-s shock of 0.5 mA was applied on the grid. Subsequently, the animals were returned to their home cages and tested for retention after a 24 h period. Retention was assessed through a step-down latency test, employing a cut-off time of 300 s. Latency, defined as the time interval during which rats descended from the platform and placed all four paws on the grid, was recorded. Any latency exceeding 300 s was capped at 300 s [39,40].

2.6. Ex vivo analysis

2.6.1. Inflammatory cytokine levels

The levels of interleukin-1 beta (IL-1 β), tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), and interleukin-10 (IL-10), in the hippocampus were quantified utilizing commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits, following the manufacturer's instructions (Sigma-Aldrich®; St. Louis, MO, USA). Cytokine concentrations were normalized to the protein concentration present in the samples. The results are presented in ng/mL.

2.6.2. Western blot

The Western blot analyses followed a method by Guerra et al. [41]. For sample preparation, rats hippocampus was dissected and homogenized in 300 μ L of ice-cold buffer A (10 mM KCl, 2 mM MgCl₂, 1 mM EDTA, 1 mM NaF, 10 μ g/mL aprotinin, 10 mM β -glycerolphosphate, 1 mM PMSF, 1 mM DTT, and 2 mM sodium orthovanadate in 10 mM HEPES, pH 7.9). After homogenization, the samples were incubated for 15 min at 0 °C and then centrifuged at 16,000 $\times$ g for 45 min at 4 °C. The supernatant was collected for cytosolic proteins, and the pellet was resuspended. To access the nuclear fraction, the pellet was resuspended in 150 μ L of ice-cold buffer B (10 mM KCl, 2 mM MgCl₂, 1 mM EDTA, 1 mM NaF, 10 μ g/mL aprotinin, 10 mM β -glycerolphosphate, 1 mM PMSF, 1 mM DTT, 2 mM sodium orthovanadate, and 1% Triton-X in 10 mM HEPES, pH 7.9). Samples were incubated for 15 min at 0 °C and then centrifuged at 16,000 $\times$ g for 45 min at 4 °C. After centrifugation, the supernatant was discarded, and the pellet was resuspended in 100 μ L of ice-cold buffer C (50 mM KCl, 2 mM MgCl₂, 1 mM EDTA, 1 mM NaF, 10 μ g/mL aprotinin, 10 mM β -glycerolphosphate, 1 mM PMSF, 1 mM DTT, 2 mM sodium orthovanadate, 420 mM NaCl, and 25% glycerol in 20 mM HEPES, pH 7.9). The samples were incubated for 15 min at 0 °C and then centrifuged at 16,000 $\times$ g for 45 min at 4 °C, and the remaining supernatant was collected and considered the nuclear fraction. Protein concentration was determined to ensure equivalent protein amounts for cytosol (80 μ g) and nucleus (20 μ g) in all samples [42]. Finally, 0.2 vol of concentrated loading buffer (200 mM Tris, 10% glycerol, 2% SDS, 2.75 mM β -mercaptoethanol, and 0.04% bromophenol blue) were added to the samples and incubated at 100 °C for 5 min.

In electrophoresis, proteins were separated on 12% sodium dodecyl sulfate–polyacrylamide gels (SDS-PAGE) for approximately 3 h. Subsequently, the gel was transferred onto nitrocellulose membranes using the Transfer-Blot® Turbo™ Transfer System (1.0 A; 30 min). The membranes were stained with 0.5% Ponceau to confirm the transfer process. After blocking for 2 h with 1% bovine serum albumin (BSA) in 0.05% Tween 20 in saline Tris–borate (TBS-T), the blots were incubated overnight with specific primary polyclonal antibodies (Table 2). The blots were washed three times with 0.05% TBS-T, followed by incubation with secondary antibodies conjugated with horseradish peroxidase (1:5000, anti-rabbit IgG or anti-mouse IgG-HRP; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) for 2 h at room temperature. Blots were developed with 3,3',5,5'-tetramethylbenzidine (Sigma-Aldrich®; St. Louis, MO, USA), and membrane quantification was performed using

Table 2

Antibodies used in protein immunoblotting.

Primary antibody	Dilution	Host	Manufacturer
Anti- β -actin	1:1000	Mouse	Sigma Aldrich
Anti-Androgen Receptor	1:1000	Rabbit	Sigma Aldrich
Anti-p38 Mitogen Activated Protein Kinase (MAPK)	1:1000	Rabbit	Cell Signaling Technology
Anti-Phospho-p38 MAPK	1:1000	Rabbit	Cell Signaling Technology
Anti-Protein Kinase B (Akt)	1:1000	Rabbit	Sigma Aldrich
Anti-Phospho-Akt	1:1000	Mouse	Santa Cruz Biotechnology
Anti-Bcl-2-associated X protein (Bax)	1:1000	Mouse	Santa Cruz Biotechnology
Anti-B-cell lymphoma-2 (Bcl-2)	1:500	Mouse	Santa Cruz Biotechnology
Anti-Cleaved Caspase-3	1:500	Rabbit	Boster Bio. Technology
Anti-pro-Brain-Derived Neurotrophic Factor (pro-BDNF)	1:500	Mouse	Santa Cruz Biotechnology
Anti-BDNF	1:500	Mouse	Sigma Aldrich
Anti-Tropomyosin receptor kinase B (TrkB)	1:1000	Mouse	Santa Cruz Biotechnology
Anti-Cyclic-AMP Response Element Binding (CREB)	1:1000	Mouse	Santa Cruz Biotechnology
Anti-Phospho-CREB	1:1000	Mouse	Santa Cruz Biotechnology

ImageJ software (NIH, Bethesda, MD, USA). The results were normalized by arbitrarily setting the ratio between the protein band and β -actin loading control.

2.7. Statistical analysis

Data normality was assessed using the Shapiro-Wilk test (parametric or non-parametric), while homoscedasticity was confirmed through Bartlett's and F-tests. Step-down latency underwent a non-parametric Scheirer-Ray-Hare extension bidirectional analysis to determine significant group differences, and the data are represented as median $\pm$ interquartile range. In all other analyzes, a parametric two-way analysis of variance (ANOVA) was conducted following the classic multifactorial model $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}$. In this model, Y_{ijk} represents the specific response variable influenced by the levels of EXE and YK11, μ denotes the overall mean of the response variable, irrespective of the distinct groups defined by EXE and YK11 levels, α_i are the fixed effect of the i -th EXE level, illustrating how the response variable's mean differs among groups defined by various EXE levels. Similarly, β_j represents the fixed effect of the j -th YK11 level, indicating mean differences among groups with varying YK11 levels. The $(\alpha\beta)_{ij}$ term captures the interaction between the i -th level of the EXE factor and the j -th level of the YK11 factor, elucidating how the effects of EXE and YK11 mutually influence the response variable. Lastly, ϵ_{ijk} serves as a random error term associated with each observation, accounting for unexplained variation by the main effects and the interaction between EXE and YK11. Tukey's post-hoc test was employed to assess group differences, with significance considered at $p < 0.05$. Data is presented as the mean $\pm$ standard error of the mean (S.E.M).

3. Results

3.1. Rat simulation pharmacokinetic profile indicates enhanced brain permeation to YK11

Our simulation of the *in silico* pharmacokinetic mechanism of YK11, based on the weight of the YK11-supplemented group (Fig. 2A), revealed remarkable permeability to muscle tissue, as expected, along with a significant ability to traverse the blood-brain barrier during the 24 h metabolic period. In Fig. 2B, based on the *in silico* pharmacokinetic profile, we estimated an increase in YK11 plasma concentration within

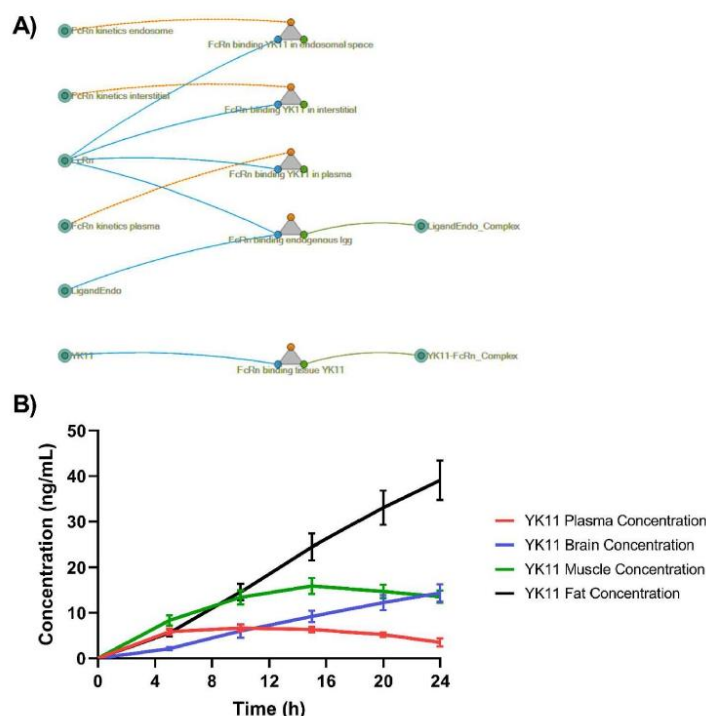


Fig. 2. Development of a full PBPK model: Computational simulation of 0.35 g/kg YK11 administration in a Wistar rat model using PK-SIM software. A) Pharmacokinetic diagram of YK11 used in PBPK model; B) YK11 pharmacokinetic simulation represented as red line: YK11 plasma concentration, blue line: YK11 brain concentration, green line: YK11 muscle concentration, black line: YK11 fat concentration, in a 24 h metabolic period. Data derived from YK11 group mean rat weights at each time-point observed and considering a 24 h supplementation frequency (once a day, daily). Values are represented as mean $\pm$ S.E.M (n = 6).

approximately 9–15 h, like that observed in striated muscle tissue. YK11 exhibits high permeability to muscle, however, its levels appear to reduce 16 h after administration. In contrast, YK11 exhibits high permeability in the brain, accompanied by a reduction in its plasma concentration. Over the 24 h period, a consistent trend in the concentration of YK11 in brain tissue are recorded. As expected, due to its steroidal character, YK11 has a high capacity for bioaccumulation in adipose tissue.

Using population *in silico* pharmacokinetic analysis, the average weight of YK11-supplemented rats serves as a demonstrative factor (Table 3). YK11 brain concentration exhibited a C_{max} of 14.39 ng/mL, comparable to YK11 muscle concentration, with C_{max} of 15.99 ng/mL, and YK11 fat tissue concentration, with C_{max} of 39.08 ng/mL. The recorded C_{max} of YK11 plasma was 6.67 ng/mL. Notably, YK11 brain concentration reached t_{max} at 24 h, while YK11 muscle concentration reached t_{max} at 15.5 h, indicative of high permeability during extended

observation periods (likely enhanced brain permeability after 24 h). The t_{max} for YK11 fat tissue concentration reached the 24 h analysis, and YK11 plasma reached a t_{max} of 9.2 h. However, owing to YK11 high affinity for muscle androgen receptors, it presented an AUC of 16.94 $\mu\text{g min/mL}$, nearly half more of that observed in YK11 in the brain, 10.02 $\mu\text{g min/mL}$. YK11 plasma reached an AUC of 7.64 $\mu\text{g min/mL}$, and as expected, YK11 in fat tissue has a higher AUC of 26.83 $\mu\text{g min/mL}$. The pharmacokinetic profile of YK11 in plasma, brain, striated muscle, and adipose tissue estimated at periods of 1 week and 5 weeks (the total duration of the experiment), was also analyzed, considering daily compound supplementation; All these results are shown in supplementary material (Fig. S1, Tables S1–S2). This analysis supports the possibility of YK11 exhibiting increased permeability in the brain, greater than muscle, over longer metabolic periods.

3.2. YK11 has high binding affinity to 5 α -Reductase type II (5 α R2)

Computational molecular docking analyses were conducted to assess the potential inhibitory effect of YK11 on 5 α R2. For this purpose, interaction analyses were performed with finasteride as a positive control for 5 α R2 inhibition and testosterone as a negative control for 5 α R2 inhibition. In the finasteride-5 α R2 complex (Fig. 3A; Table 4), a ΔG_{bind} of $-7.5 \text{ kcal/mol}^{-1}$ was observed, highlighting alkyl interactions with Ala24 (4.66 Å), Ala117 (4.37 Å), and notably Arg114 (4.46 Å), a key inhibitory residue site of 5 α R2. In the YK11-5 α R2 complex (Fig. 3B; Table 4), a ΔG_{bind} of $-9.5 \text{ kcal/mol}^{-1}$ was observed, with π -alkyl interactions in Phe223 (4.40 Å) and hydrogen interactions with Arg114

Table 3
Pharmacokinetic parameters based in the mean weight of YK11 rats after PBPK simulation in 24 h metabolic period.

	C_{max} (ng/mL)	t_{max} (h)	AUC ($\mu\text{g min/mL}$)
Plasma concentration	6.67 ± 0.27	9.2 ± 0.18	7.64 ± 0.33
Brain concentration	14.39 ± 0.75	24.0 ± 0	10.02 ± 0.55
Muscle concentration	15.99 ± 0.67	15.5 ± 0.14	16.94 ± 0.74
Fat concentration	39.08 ± 1.68	24.0 ± 0	26.83 ± 1.22

Values are represented as mean $\pm$ S.E.M (n = 6).

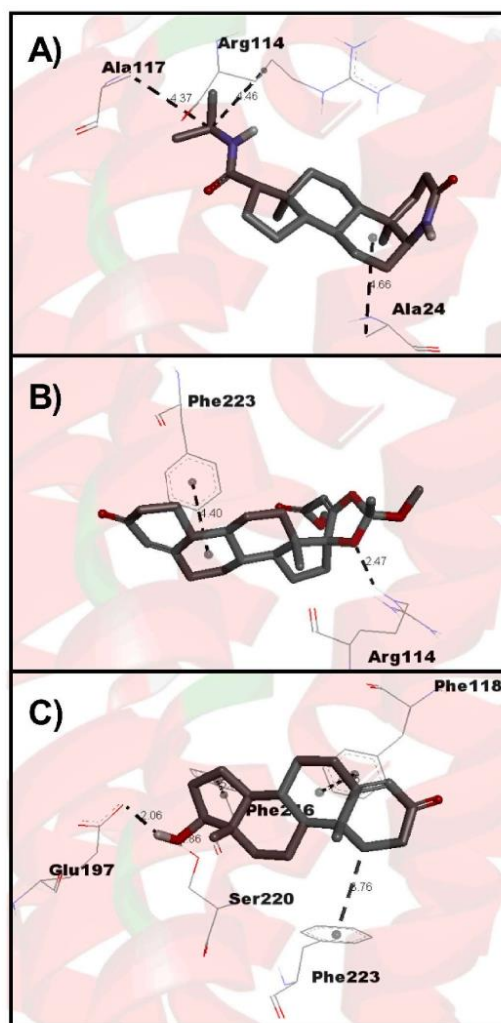


Fig. 3. Interaction between 5 α R2 with finasteride, YK11 and testosterone on molecular docking studies. A) Finasteride-5 α R2 complex; B) YK11-5 α R2 complex; C) Testosterone-5 α R2 complex. Data obtained using the software AutoDockVina, AutoDockTools and Discovery Studio Visualizer.

Table 4
Predicted ΔG_{bind} and distance from Arg114 site from finasteride, YK11 and testosterone bound with 5 α R2 on molecular docking simulations.

Ligand	ΔG (kcal mol ⁻¹)	Arg114 distance (Å)
Finasteride	-7.5	4.46
YK11	-9.5	2.47
Testosterone	-10.4	>10

(2.47 Å). In the testosterone-5 α R2 complex (Fig. 3D; Table 4), a ΔG_{bind} of -10.4 kcal/mol⁻¹ was noted, highlighting π -alkyl interactions with Phe118 (4.75 Å) and Phe216 (5.33 Å), hydrogen interactions with

Glu197 (2.06 Å) and Ser220 (2.86 Å), and a π -sigma interaction with Phe223 (3.76 Å). Interactions with Arg114 were limited to van der Waals interactions, with a low or negligible probability of interaction (>10 Å; interaction not shown in 3D visualize tool). 2D representations of the finasteride-5 α R2, YK11-5 α R2, and testosterone-5 α R2 complexes are shown in supplementary material (Fig. S2).

3.3. YK11 demonstrates high conformational stability at the 5 α R2 binding site

To assess conformational stability in the ligand-protein complexes finasteride-5 α R2, YK11-5 α R2, and testosterone-5 α R2, we conducted molecular dynamics simulations for 50 ns (Fig. 4). Our results revealed a similar average fluctuation between structures complexed with 5 α R2, with an average of 3.27 Å for finasteride, 3.62 Å for testosterone and 3.89 Å for YK11. Of importance, testosterone exhibited a higher conformational instability observed towards the end of the simulation, between 40 and 50 ns, where the average fluctuation up to 5.05 Å, in contrast with finasteride (3.35 Å) and YK11 (3.93 Å), at the same time. Based on these findings, it can be inferred that YK11 exhibits conformational stability like finasteride when in complex with 5 α R2, suggesting their potential capacity for enzyme site competition.

3.4. YK11 does not affect spontaneous locomotion activity

In the OFT results, no statistically significant differences were observed in the parameters of spontaneous activity, including distance traveled, rearing number, or velocity (Table 5).

3.5. YK11 reduces aversive memory retention regardless of EXE

The step-down latencies in the inhibitory avoidance task are depicted in Fig. 5. The results for aversive memory acquisition reveal no statistically significant differences among the experimental groups (Fig. 5A). However, aversive memory retention results 24 h after acquisition (Fig. 5B) showed a decrease in the YK11 group compared to the Control ($p = 0.0252$) and the EXE ($p = 0.0002$). Additionally, a decrease in aversive memory retention was observed in the EXE + YK11 group compared to the Control ($p = 0.0437$) and the EXE ($p = 0.0010$). Of importance, YK11 and EXE + YK11 exhibited a reduction in step-down latency of approximately 90% and 75%, respectively, when compared to the Control group.

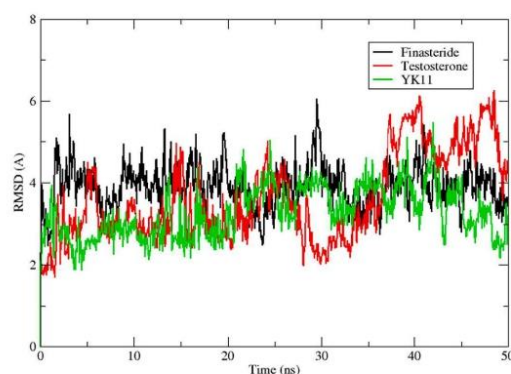


Fig. 4. RMSD plots of the backbone for the complexes Finasteride (black line); Testosterone (red line); YK11 (green line) in the active site of 5 α R2 during 50 ns simulation.

Table 5

Effects of YK11 administration associated with swimming exercise in the spontaneous locomotor activity accessed with OFT.

Group	Distance (mm)	Rearing number	Velocity (mm/s)
Vehicle	6420 ± 1277	20.00 ± 4.258	21.04 ± 4.823
YK11	7150 ± 782.5	20.80 ± 5.919	21.52 ± 1.828
EXE	8067 ± 1474	18.80 ± 1.881	22.72 ± 1.605
EXE + YK11	5491 ± 1291	17.20 ± 2.728	21.45 ± 4.044

Values are expressed as mean ± S.E.M (n = 5–6/group).

3.6. YK11 enhances the nuclear-cytoplasmic translocation of hippocampal androgen receptor (AR)

The western immunoblotting representative membranes of

hippocampal AR, both cytosolic and nuclear fractions are shown in Fig. 6A. AR levels in the cytosolic fraction of the hippocampus (Fig. 6B) were decreased in YK11 when compared to the Control group ($p = 0.0008$), while EXE had an increase in cytosolic AR levels when compared to Control ($p = 0.0204$) and EXE + YK11 ($p < 0.0001$). AR levels in nuclear fraction of the hippocampus (Fig. 6C) were increased in YK11 when compared to the Control group ($p = 0.0015$), and in EXE + YK11 concerning to Control ($p = 0.0010$). These results reveal the potential activation of AR at the nuclear level by YK11, demonstrating its capacity to increase the translocation of AR from the cytosol to the nucleus.

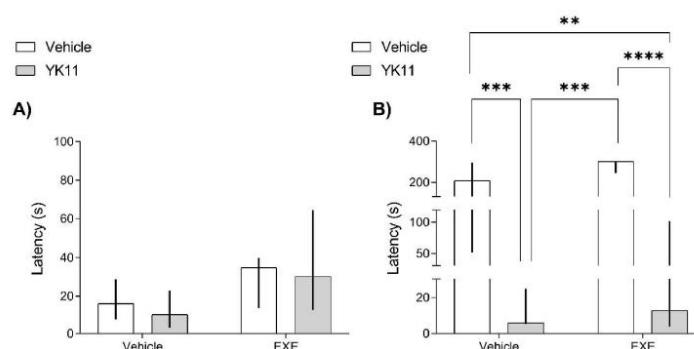


Fig. 5. Effects of YK11 and swimming exercise on step-down latency. A) Training session (memory acquisition); B) Test sessions (memory evocation). Values are presented as median with $\pm$ interquartile range (n = 5–6/group), and statistical significance was determined using bidirectional non-parametric Scheirer-Ray-Hare test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

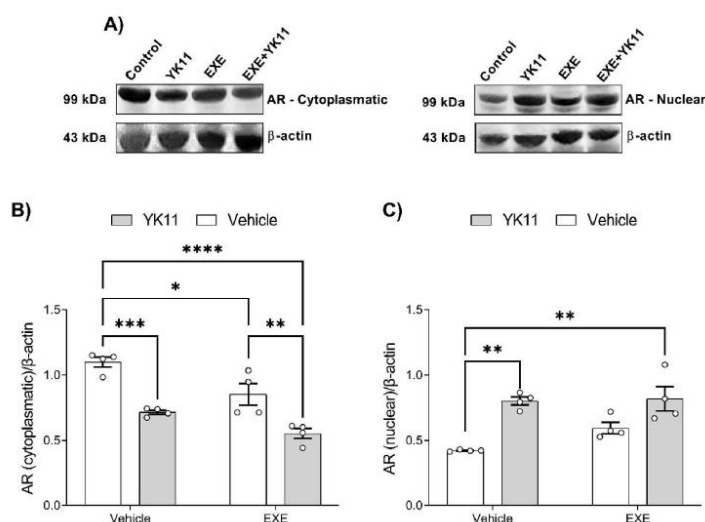


Fig. 6. Effects of YK11 and swimming exercise on hippocampal AR levels in nuclear and cytosolic fractions. A) Representative immunoblotting of cytosolic and nuclear AR fractions; B) Immunoreactivity of AR (cytoplasmic); C) Immunoreactivity of AR (nuclear); β -actin was utilized as a loading control. Values are presented as mean with $\pm$ S.E.M (n = 4), and statistical significance was determined using Two-Way Analysis of Variance (ANOVA) followed by Tukey's post hoc test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

3.7. YK11 modifies neurobiological mechanisms of memory consolidation

Western immunoblotting representative membranes for proBDNF, BDNF, TrkB, CREB/Phospho-CREB are presented in Fig. 7A. In proBDNF levels (Fig. 7B), no statistically significant differences were observed between the experimental groups. In BDNF levels (Fig. 7C), a decrease was observed in the YK11 when compared to the Control ($p = 0.0134$) and the EXE ($p = 0.0035$). Similarly, a reduction in BDNF levels were exhibited in the EXE + YK11 compared to the Control ($p = 0.0159$) and the EXE ($p = 0.0041$). In TrkB levels (Fig. 7D), there was a decrease

in the YK11 group compared to the Control ($p = 0.0005$) and the EXE ($p < 0.0001$), mirroring the reduction in TrkB levels in the EXE + YK11 when compared to the Control ($p = 0.0029$) and the EXE ($p = 0.0003$).

Regarding the nuclear hippocampal fraction, it was observed that total CREB levels (Fig. 7E) did not exhibit statistically significant differences between the experimental groups. However, phospho-CREB (Fig. 6F) was reduced in the YK11 group compared to the Control ($p < 0.0001$) and the EXE ($p < 0.0001$). A reduction was also showed in phospho-CREB levels in the EXE + YK11 compared to the Control ($p = 0.0005$) and the EXE ($p < 0.0001$), while the latter displayed an increase

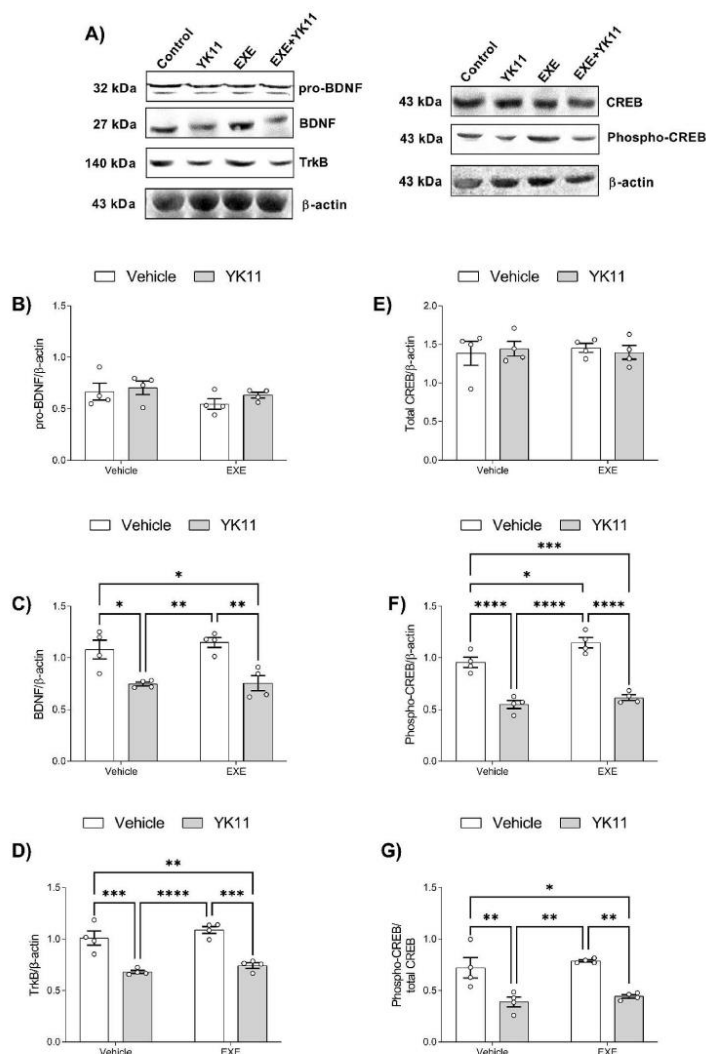


Fig. 7. Effects of YK11 and swimming exercise on hippocampal memory consolidation proteins proBDNF, BDNF, TrkB in cytosolic fraction, and CREB and their respective phosphorylated form, both last in nuclear fraction. A) Representative immunoblotting of proBDNF, BDNF, TrkB, CREB and phospho-CREB; B) Immunoreactivity of proBDNF; C) Immunoreactivity of BDNF; D) Immunoreactivity of TrkB; E) Immunoreactivity of CREB; F) Immunoreactivity of phospho-CREB; G) Phospho-CREB/total CREB ratio. β-actin was utilized as a loading control. Values are presented as mean with $\pm$ S.E.M ($n = 4$), and statistical significance was determined using Two-Way Analysis of Variance (ANOVA) followed by Tukey's post hoc test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

compared to the Control ($p = 0.0350$). In the phospho-CREB/total CREB ratio (Fig. 7G), YK11 exhibited a reduction compared to the Control ($p = 0.0060$) and the EXE ($p = 0.0015$), and EXE + YK11 also displayed a reduction when compared to the Control ($p = 0.0194$) and the EXE ($p = 0.0046$). Of note, YK11 and EXE + YK11 exhibited a reduction on the phospho-CREB/total CREB ratio in approximately 50% and 40%, respectively, when compared to the Control group, shedding light on possible changes in step-down latency.

3.8. YK11 increases inflammation in the hippocampus

In IL-1 β levels (Fig. 8A), YK11 exhibited an increase compared to the Control group ($p = 0.0005$) and the EXE group ($p = 0.0043$). Furthermore, IL-1 β levels were elevated in the EXE + YK11 group when compared to the Control group ($p = 0.0002$) and the EXE group ($p = 0.0015$). As for TNF- α levels (Fig. 8B), no statistically significant differences were observed. In the IL-6 levels (Fig. 8C), an increase was evident in the YK11 group compared to the Control group ($p < 0.0001$), the EXE group ($p < 0.0001$), and the EXE + YK11 group ($p < 0.0001$). Regarding IL-10 levels (Fig. 8D), an increase was observed in the YK11 group when compared to the Control group ($p = 0.0001$) and the EXE group ($p < 0.0001$). Also, IL-10 levels were elevated in the EXE + YK11 group concerning the Control ($p = 0.0057$) and the EXE group ($p < 0.0001$). Lastly, it's worth noting a reduced IL-10 levels in the EXE group compared to the Control ($p = 0.0015$), demonstrating a neuroprotective effect.

3.9. YK11 increases the recruitment of apoptotic proteins

Western immunoblotting representative membranes for p38 MAPK and Akt are shown in Fig. 9A. There were no statistically significant differences in total p38 MAPK levels (Fig. 9B). In terms of phospho-p38 MAPK levels (Fig. 9C), an increase was observed in the YK11 group when compared to the Control group ($p = 0.0051$) and the EXE group ($p = 0.0385$). Additionally, an increase in phospho-p38 MAPK was displayed in the EXE + YK11 when compared to the Control group ($p = 0.0038$) and the EXE group ($p = 0.0284$). Also, the phospho-p38 MAPK/total p38 MAPK ratio (Fig. 9D) is increased in the YK11 group compared to the Control group ($p = 0.0100$) and the EXE group ($p = 0.0235$). Furthermore, an increase in the phospho-p38 MAPK/total p38 MAPK ratio was exhibited in the EXE + YK11 when compared to the Control ($p = 0.0073$) and the EXE group ($p = 0.0172$). Notably, no statistically significant differences were observed in the levels of total Akt (Fig. 9E), phospho-Akt (Fig. 9F), and the phospho-Akt/total Akt ratio (Fig. 9G).

Western immunoblotting representative membranes for Bax, Bcl-2, and cleaved caspase-3 are presented in Fig. 10A. In Bax levels (Fig. 10B), an increase was observed in the YK11 group when compared to the Control group ($p = 0.0035$), and the EXE group ($p = 0.0001$). Similarly, an increase in Bax levels were found in the EXE + YK11 when compared to the Control group ($p = 0.0214$) and the EXE group ($p = 0.0005$). In Bcl-2 levels (Fig. 10C), a decrease was verified in the YK11 compared to the Control ($p = 0.0011$), the EXE ($p < 0.0001$), and the EXE + YK11 ($p = 0.0151$). An increase in Bcl-2 levels was observed in the EXE group compared to the Control ($p = 0.0362$), indicating a neuroprotective effect. Cleaved caspase-3 levels (Fig. 10D) were found

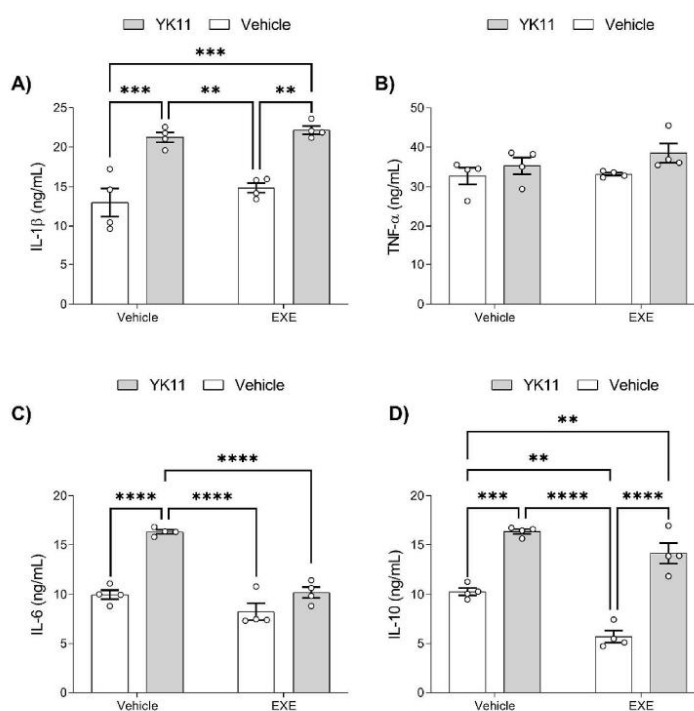


Fig. 8. Effects of YK11 and swimming exercise on hippocampal inflammatory biomarkers levels. A) IL-1 β levels; B) TNF- α levels; C) IL-6 levels; D) IL-10 levels. Values are expressed as means with $\pm$ S.E.M. ($n = 4$), and statistical significance was determined using Two-Way Analysis of Variance (ANOVA) followed by Tukey's post hoc test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

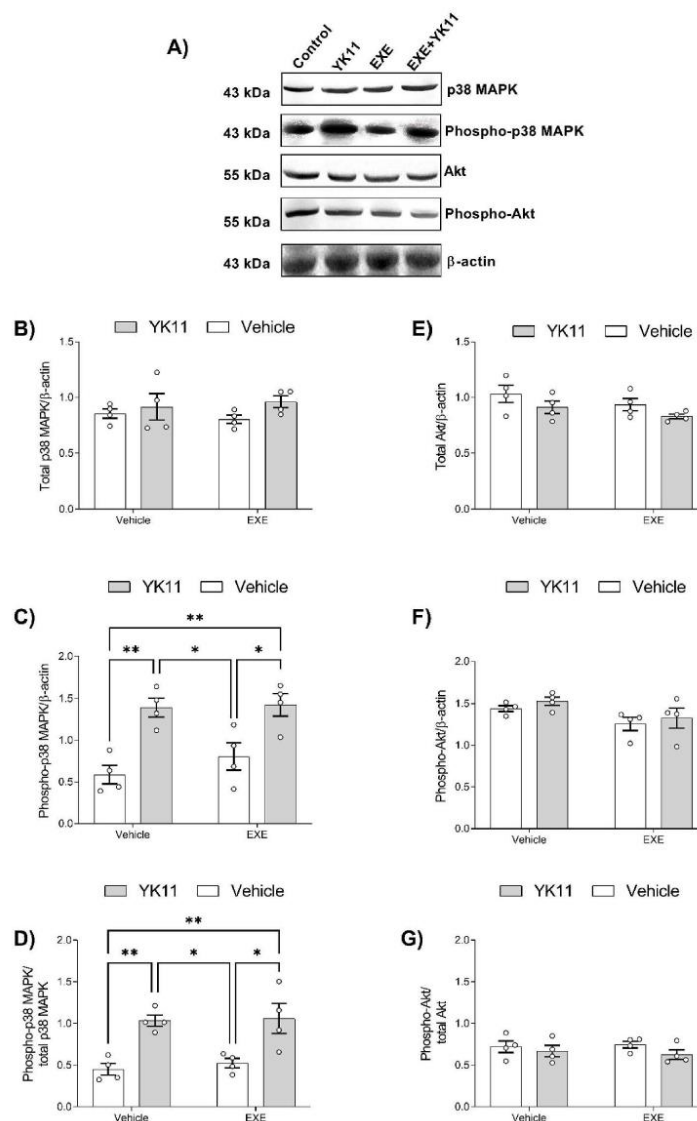


Fig. 9. Effects of YK11 and swimming exercise in hippocampal pro-apoptotic factors p38 MAPK and Akt. A) Representative immunoblotting of p38 MAPK and Akt, and their respective phosphorylated forms; B) Immunoreactivity of total p38 MAPK; C) Immunoreactivity of phospho-p38 MAPK; D) Phospho-p38 MAPK/total p38 MAPK ratio; E) Immunoreactivity of total Akt; F) Immunoreactivity of phospho-Akt; G) Immunoreactivity of phospho-Akt/total Akt. β-actin was utilized as a loading control. Values are presented as mean with $\pm$ S.E.M (n = 4), and statistical significance was determined using Two-Way Analysis of Variance (ANOVA) followed by Tukey's post hoc test (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

to be elevated in the YK11 group when compared to the Control ($p = 0.0012$), the EXE ($p < 0.0001$), and the EXE + YK11 group ($p = 0.0283$). Consistent with previous findings, the EXE group demonstrated a neuroprotective effect as cleaved caspase-3 levels were reduced compared to the Control group ($p = 0.0049$).

4. Discussion

Our study delves into the mechanisms of YK11 within the hippocampus, revealing its significant influence on neurochemical function. YK11, a SARM, acts in the hippocampus and modulates the AR, impacting memory, neuroinflammation, and apoptosis pathways. These effects occur mostly irrespective of EXE, a common combination among

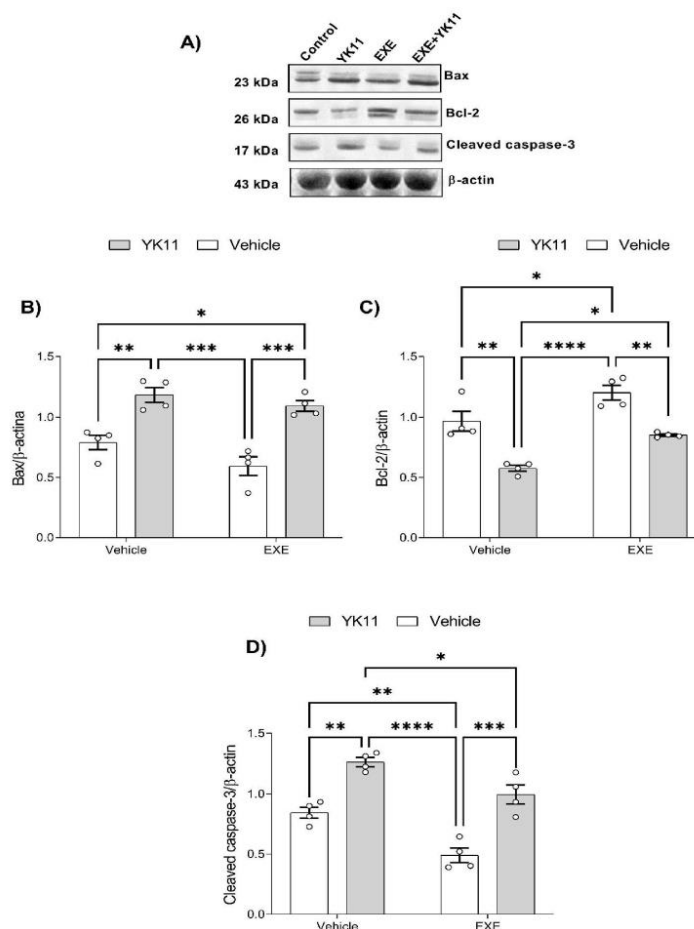


Fig. 10. Effects of YK11 and swimming exercise in hippocampal pro-apoptotic factors Bax, bcl-2 and cleaved-caspase 3. A) Representative immunoblotting of bax, bcl-2 and cleaved-caspase 3; B) Immunoreactivity of bax; C) Immunoreactivity of bcl-2; D) Immunoreactivity of cleaved-caspase 3. β-actin was utilized as a loading control. Values are presented as mean with $\pm$ S.E.M (n = 4), and statistical significance was determined using Two-Way Analysis of Variance (ANOVA) followed by Tukey's post hoc test (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

SARMs users. Initially, we investigate the metabolic mechanisms of YK11 through computational simulations, focusing on its interactions with 5 α R2, a crucial enzyme in SARMs metabolism. Furthermore, we aim to elucidate YK11 binding capacity with hippocampal AR and its impact on aversive memory behavior, coupled with modulations in the pro-BDNF/BDNF/TrkB/CREB pathway. Finally, our examination extends to its role in inflammatory cytokines and their influence in p38 MAPK/Akt/Bax/Bcl-2/caspase-3 pro-apoptotic signaling.

Firstly, we examined the YK11 pharmacokinetics, specifically its ability to permeate the brain and cross the blood-brain barrier (BBB), as previously documented in the literature by similar SARMs [43]. For that, our study employs *in silico* PBPK modeling, utilizing default parameters for rat metabolism and skeletal muscle tissue as a reference, due to the high action of SARMs in muscle tissues [7]. Our findings unveiled a rapid increase in YK11 permeability within skeletal muscle, followed by a subsequent decline after 15 h post-supplementation. In contrast, YK11 concentration in the brain exhibited a sustained and consistent increase,

showing a remarkable ability to permeate the blood-brain barrier over 24 h. Additionally, YK11 displayed a notable capacity for bioaccumulation in less vascularized compartments like adipose tissue, elevating its bioavailability, possibly through all experiment. This accumulation pattern raises concerns, particularly given the substance's heightened permeability in the brain. These observations align with Akita et al. [44] findings on NEP28, a non-steroidal SARM, who underscores the prominent role of SARMs in muscle and brain compared to other tissues. Based on our five-week daily YK11 supplementation, we suggest a potential for higher brain concentrations than muscle tissue, raising concerns about chronic YK11 use, particularly at high anabolic doses.

Given the remarkable ability of SARMs to permeate the CNS, it is crucial to explore the pharmacodynamic within their tissues. The key determinants of this metabolic landscape predominantly hinge on their interplay with 5- α -reductase enzymes [45,46]. These enzymes wield substantial influence over the metabolic conversion of testosterone and

its derivatives, catalyzing the reduction of double bonds situated between the 4th and 5th carbon atoms in their structure [47]. This reaction form dihydrotestosterone (DHT), the biologically active variant testosterone derivatives. While this reduced form showcases heightened affinity for AR, thereby accentuating its anabolic potential, it concurrently imposes a notable drawback in full testosterone levels [48].

SARMs play an essential role in the AR metabolism, while they have little to no metabolism by these enzymes, acting directly on AR [49], some of them exhibit antagonistic potential with 5- α -reductase enzymes, specifically with 5- α -reductase type II (5 α R2), competitively inhibiting their activation site [45]. We explored the interaction of YK11 with 5 α R2 through molecular docking and dynamics, using finasteride (positive control) and testosterone (negative control) for comparison. YK11 exhibited a high potential for 5 α R2 interaction, with a predicted ΔG_{bind} of $-9.5 \text{ kcal mol}^{-1}$, similar to testosterone with ΔG_{bind} $-10.4 \text{ kcal mol}^{-1}$. In comparison, finasteride also has a high capacity for interaction with 5 α R2, despite a lower predicted ΔG_{bind} of $-7.5 \text{ kcal mol}^{-1}$ compared to testosterone and YK11. Analysis revealed all three substances could bind to 5 α R2, with interaction at Arg114 residues determining some of them inhibitory potential [24]. Testosterone exhibited lower inhibitory potential, attributable to its distance exceeding $10 \text{ \AA}$ from Arg114. In contrast, finasteride has alkyl interaction at $4.46 \text{ \AA}$ with Arg114, displayed elevated inhibitory potential. Notably, YK11, forming a hydrogen bond at $2.47 \text{ \AA}$ with Arg114, demonstrated a greater interaction potential than finasteride. Notably, hydrogen bonds have greater strength than alkyl interactions, due to the directional nature of partial charge transfer [50], in this case, between the oxygen molecules of YK11. These results are corroborated when we observe the dynamics of these formed complexes, where the YK11-5 α R2 complex presents a stable fluctuation at the 5 α R2 pocket site, compared to finasteride and testosterone, even though the average fluctuation (RMSD = $3 \text{ \AA}$) is similar among all tested structures, given the high potential for interaction with 5 α R2. Notably, our study identified Phe223 significance in YK11-5 α R2 and testosterone-5 α R2 complexes, absent in finasteride-5 α R2. In studies investigating molecular stability within complexes involving 5 α R2, Saah et al. (2023) demonstrates that certain sites, distinct from those associated with the enzyme's inhibitory potential, contribute to its stability. Among these, our study highlights the significance of the Phe223 residue in the complexes formed between YK11-5 α R2 and testosterone-5 α R2, a result not observed in finasteride-5 α R2 complex.

Our previous predictive simulations suggest that YK11, in contrast to testosterone derivatives, may exhibit increased bioavailability, particularly influencing AR [11]. This aligns with our experimental model, where we observed elevated nuclear AR levels in the hippocampus of rats treated with YK11 and EXE + YK11. This rise is likely associated to reduced cytoplasmic AR levels, indicating the binding and translocation of the YK11-AR complex to the nucleus. Importantly, YK11 is presumed to interact directly with AR for this metabolic impact [9]. As noted earlier, YK11 potentially inhibits the 5 α R2 enzyme, suggesting that its anabolic potential is closely tied to YK11 supplementation, limiting DHT formation by potentially decreasing 5 α R2 activity. This hypothesis is supported by our observations in the EXE group, where despite a reduction in cytosolic AR levels, no corresponding translocation occurred at the nuclear level. This underscores YK11 importance in inhibiting 5 α R2 to enhance SARMs anabolic potential. Collectively, YK11 inhibitory potential, involving hydrogen bonds with Arg114 and π -alkyl interactions with Phe223 in 5 α R2, along with its high binding activity to nuclear AR in the hippocampus, implies this SARM as potent anabolic agent with significant potential to permeate and function effectively within the hippocampus.

In this sense, YK11 demonstrates significant potential in accessing brain regions, particularly through the modulation of hippocampal AR. The hippocampus, crucial for memory formation, consolidation, and evocation, plays a pivotal role in neuroplasticity [22]. The observed impact of YK11 on hippocampal AR suggests a potential modification in

memory-related behaviors in animals. Our step-down latency results indicate that, regardless of the combination with EXE, YK11 can indeed influence the animals' memory retention ability 24 h post-task training. Thus, it is plausible to infer that YK11 action in the hippocampus, primarily mediated by its binding to AR, could affect the consolidation capacity of aversive memory in these animals. In this sense, investigating the neurobiological mechanisms related to memory is valid for understanding the extent of such impairments. Our results demonstrate that YK11 was able to decrease BDNF levels in the hippocampus of rats, an important neurotrophin that plays fundamental roles in neuroplasticity and neuronal survival [51]. Furthermore, YK11 was able to reduce TrkB levels, which, when associated with its ligand BDNF, exert pleiotropic functions in neuronal differentiation and growth ([52]; Camuso et al., 2022). Together, the BDNF/TrkB signaling is fundamental for synaptic plasticity and early- and late-phase long-term potentiation (LTP) in hippocampal CA1 [51]. These alterations were observed regardless of the association of YK11 with EXE, a factor extensively associated with BDNF-mediated neuroplasticity (Murawska-Ciałowicz et al., 2021), raising concerns about the effects of abusive anabolic doses of YK11. Notably, these alterations in the BDNF/TrkB signaling appear to be largely mediated by the action of YK11 in the hippocampus itself, as there were no changes in the levels of pro-BDNF ($\sim 32 \text{ kDa}$ immature form), an important protein that regulates the signaling of mature BDNF [53].

Building upon our findings, we delved deeper into memory mechanisms, with a specific focus on CREB levels, a protein that effectively acts in memory consolidation [41,54]. Our results demonstrate that YK11 can downregulate CREB levels, regardless of its combination with EXE, aligning with earlier observations in the BDNF/TrkB pathway. The intricate interplay of the BDNF/TrkB/CREB pathway is acknowledged as a crucial element linked to cognitive function enhancement [55]. Perturbations in these mechanisms are strongly associated with the initiation of neurodegenerative disorders [51]. Dysfunctions in these pathways have been reported to contribute to Alzheimer's Disease (AD) development, heightening β -amyloid protein accumulation and reducing neuronal differentiation, primarily mediated by compromised BDNF/TrkB/CREB signaling [56–58]. Additionally, the literature extensively documents the impact of anabolic substance misuse on memory neurobiological mechanisms in rats, when Wood and Serpa [59] demonstrated that the abusive use of testosterone could impair objective recognition memory in rats, indicating a substantial decline in cognitive flexibility. Also, Bueno et al. [60] reported similar effects on object recognition memory, this time with the abusive use of boldenone, which, along with stanozolol, was associated with increased anxiety and depression symptoms in these animals. Our study stands as the first to unveil the effects of abusive-anabolic doses of SARMs on memory consolidation and their intricate influence on the neurobiology of the BDNF/TrkB/CREB pathway.

The dysregulation of synaptic plasticity signaling pathways, often associated with inflammatory agents [61], is influenced by disruptive elements activating BDNF/TrkB signaling impairments, commonly linked to inflammatory cytokines [62]. Our study aligns with this paradigm, revealing elevated IL-1 β and IL-6, indicating inflammation in response to YK11. Interestingly, EXE mitigates YK11 hippocampal inflammation effects, particularly on IL-6, showcasing defensive responses within the inflammation metabolism. Notably, IL-10, a crucial anti-inflammatory cytokine, significantly increases in response to YK11, both in isolation and combined with EXE, highlighting its role in resisting harmful substances. Metabolic regulation of inflammation involves Janus kinase/signal transducer and activator of transcription (JAK-STAT) receptor and Tumor Necrosis Factor Receptor 1 (TNFR1) signaling mechanisms [63]. JAK-STAT, implicated in Interleukin-2 (IL-2), Interferon-gamma (IFN- γ), and primarily IL-6 metabolism [64], is likely the pathway for YK11-induced inflammation, as TNF- α /TNFR1 levels show no significant differences. This emphasizes the complex interplay between inflammation, cytokines, and neurotrophins,

shedding light on potential therapeutic targets [65].

The intricate interplay of pro-inflammatory cytokines is pivotal for various physiological processes [66]. However, their excessive expression disrupts this delicate balance, leading the system toward chronic inflammation and often acting as a precursor to apoptosis (Bock and Riley, 2022). Given the increased levels of inflammatory cytokines in YK11, our aim was to unravel its potential impact on MAPKs proteins, established components of the apoptosis pathway ([67]; Bock and Riley, 2022). Our findings revealed elevated p38 MAPK levels in the hippocampus of rats supplemented with YK11, regardless of its combination with EXE. The activation of p38 MAPK is intricately regulated by cellular stress stimuli, known to modulate the biosynthesis of pro-inflammatory cytokines [68,69]. Therefore, in the context of altered neurochemical markers, the rise in p38 MAPK levels may indicate a protective cellular response, acting as a compensatory factor against pro-inflammatory cytokine biosynthesis and signaling a harmful stimulus [68]. Counter-regulatory mechanisms to p38 MAPK activity, mediated by Akt, are recognized as significant factors in understanding apoptotic damage in cells [70]. Despite no significant differences in Akt levels among experimental groups, our analysis suggests that the initial apoptosis modulation by YK11 in the hippocampus possibly does not involve Akt but rather implicates p38 MAPK. The substantial increase in pro-inflammatory cytokine levels may explain this, as Akt typically plays an inversely proportional role to p38 MAPK activity in acute inflammation or less severe conditions; however, in cases of chronic damage, p38 MAPK predominantly influences signaling in intrinsic mechanisms, leading to cell apoptosis (Wang et al., 2017; [67]).

The intricate interplay between pro-inflammatory cytokines and elevated p38 MAPK levels may indicate substantial damage to the intrinsic and extrinsic pathway of cellular apoptosis in the hippocampus (Wang et al., 2017). Our findings reveal an increase in the pro-apoptotic protein Bax and a decrease in the anti-apoptotic protein Bcl-2 in YK11-supplemented rats' hippocampus. The increased Bax/Bcl-2 ratio signals activation of the intrinsic apoptosis pathway, where Bax promotes mitochondrial outer membrane permeabilization, leading to caspase activation and subsequent cell death [71]. In this context, we observed elevated cleaved caspase-3 levels, the primary effector caspase in apoptosis, in the YK11-treated group. This further supports the involvement of the extrinsic pathway, as caspase-3 activation downstream of mitochondrial dysfunction aligns with Dahleh et al. [11] findings, indicating YK11 role in mitochondrial respiratory chain complex dysregulation and alterations in mitochondrial fission protein Drp1^{Ser637} and fusion proteins Mfn1/2. Interestingly, combined EXE + YK11 treatment reversed the inhibitory effect on Bcl-2 and mitigated the rise in cleaved caspase-3, suggesting EXE inherent neuroprotective effects, potentially through anti-apoptotic factor upregulation or MAPK signaling modulation [72]. However, it's crucial to emphasize the nuanced nature of these results; the absence of reduced Bax levels in the EXE + YK11 group's hippocampus underscores potential cellular apoptosis harm associated with this SARM, even with a regular exercise regimen.

5. Conclusion

Our investigation into the neurobiological effects of YK11 in the hippocampus reveals its multifaceted impact on memory, neuro-inflammation, and apoptosis pathways. YK11 unique ability to permeate the CNS, particularly the hippocampus, coupled with its inhibitory action on 5αR2, underlines its mighty anabolic potential. Computational simulations and experimental data highlight YK11 high affinity for hippocampal AR, reinforcing its role as a SARM with significant neurobiological implications. Memory-related behavioral alterations observed, coupled with disruptions in the BDNF/TrkB/CREB pathway, emphasize the importance of elucidating the intricate mechanisms underlying SARM misuse. Additionally, our findings shed light on the inflammatory responses and apoptotic pathways influenced by YK11,

particularly through the p38 MAPK/Bax/Bcl-2/caspase-3 signaling. As the prevalence of SARMS, including YK11, continues to rise, our study underscores the critical necessity for a detailed comprehension of their impact on brain function and the associated health risks.

CRedit authorship contribution statement

Mustafa Munir Mustafa Dahleh: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Vandreza Cardoso Bortolotto:** Visualization, Validation, Methodology, Investigation, Data curation. **Silvana Peterini Boeira:** Supervision, Funding acquisition, Formal analysis. **Hecson Jessor Segat:** Supervision, Formal analysis. **Gustavo Petri Guerra:** Writing – review & editing, Investigation, Funding acquisition, Formal analysis, Data curation. **Marina Prigol:** Writing – review & editing, Visualization, Validation, Supervision, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cbi.2024.110971>.

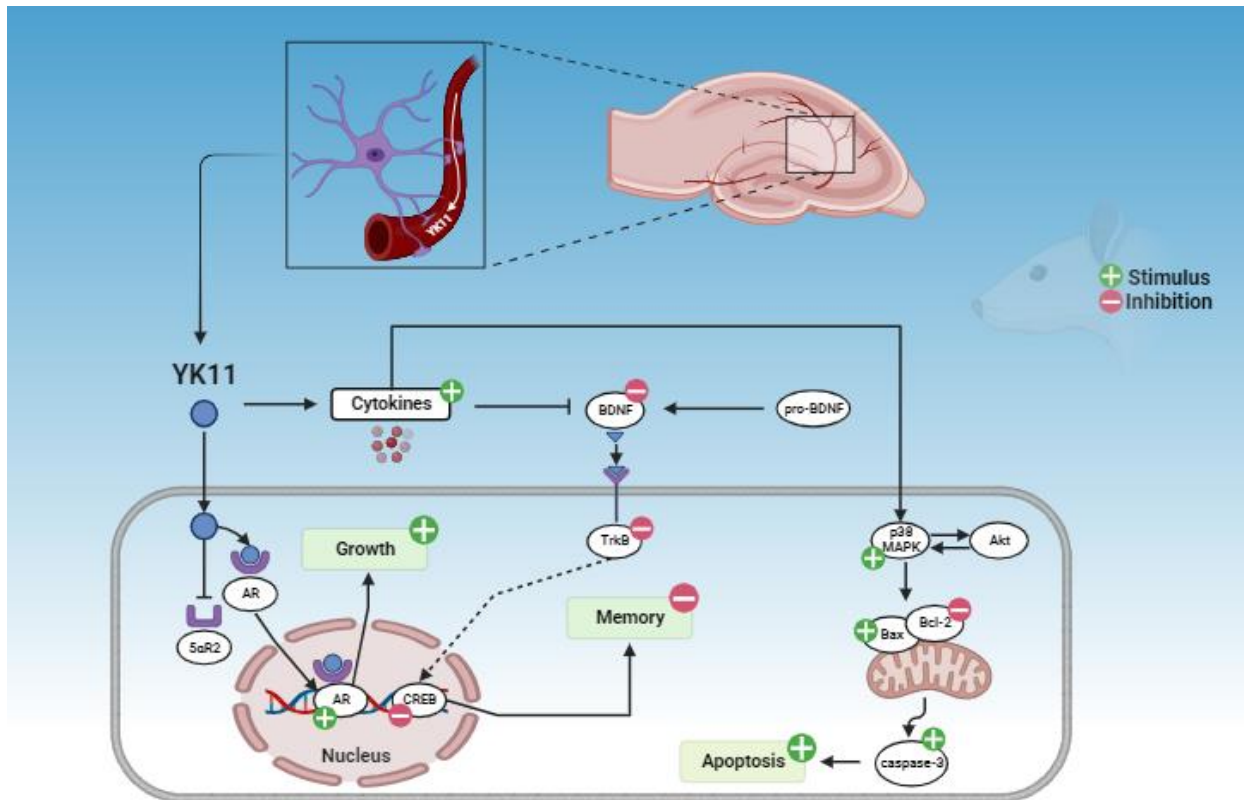
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Graphical Abstract – Artígo científico 2



5.2.1. Material suplementar: Artigo científico 2

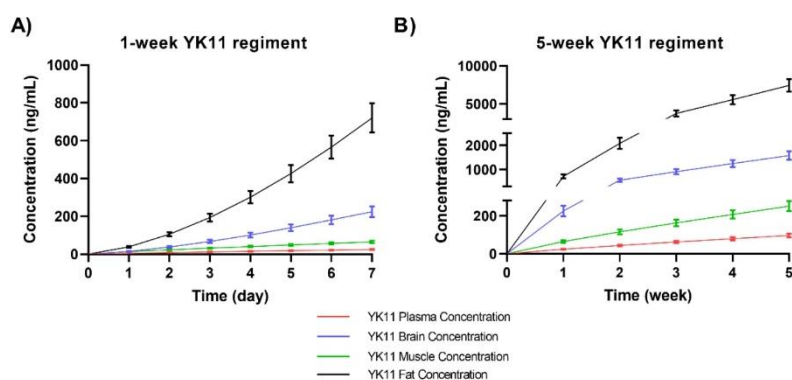


Figure S1. Pharmacokinetic profile simulation of YK11 during 1-week (A) and 5-week (B) metabolic periods. Supplementation frequency occurs every 24 hours (once a day), utilizing the actual weight of YK11-supplemented rats. The depicted curves illustrate red line - YK11 plasma concentration, blue line - YK11 brain concentration, green line - YK11 muscle concentration, black line – YK11 fat concentration. Values are represented as mean and error (n = 6).

Table S1. Pharmacokinetic parameters based in the mean weight of YK11 rats after PBPK simulation in 1-week metabolic period

	C_{max} (ng/mL)	AUC (mg·min/mL)
Plasma concentration	24.64 ± 1.022	0.1317 ± 0.004
Brain concentration	224.82 ± 10.9	0.94 ± 0.045
Muscle concentration	64.99 ± 2.682	0.358 ± 0.014
Fat concentration	720.13 ± 30.25	2.87 ± 0.12

Values are represented as mean $\pm$ S.E.M (n = 6).

Table S2. Pharmacokinetic parameters based in the mean weight of YK11 rats after PBPK simulation in 5-week metabolic period

	C_{max} (ng/mL)	AUC (mg·min/mL)
Plasma concentration	96.47 ± 4.025	2.625 ± 0.11
Brain concentration	1577.71 ± 68.9	37.54 ± 1.688
Muscle concentration	250.97 ± 25.62	6.857 ± 0.284

Fat concentration	7437.45 ± 10.46	158.1 ± 6.623
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Values are represented as mean $\pm$ S.E.M (n = 6).

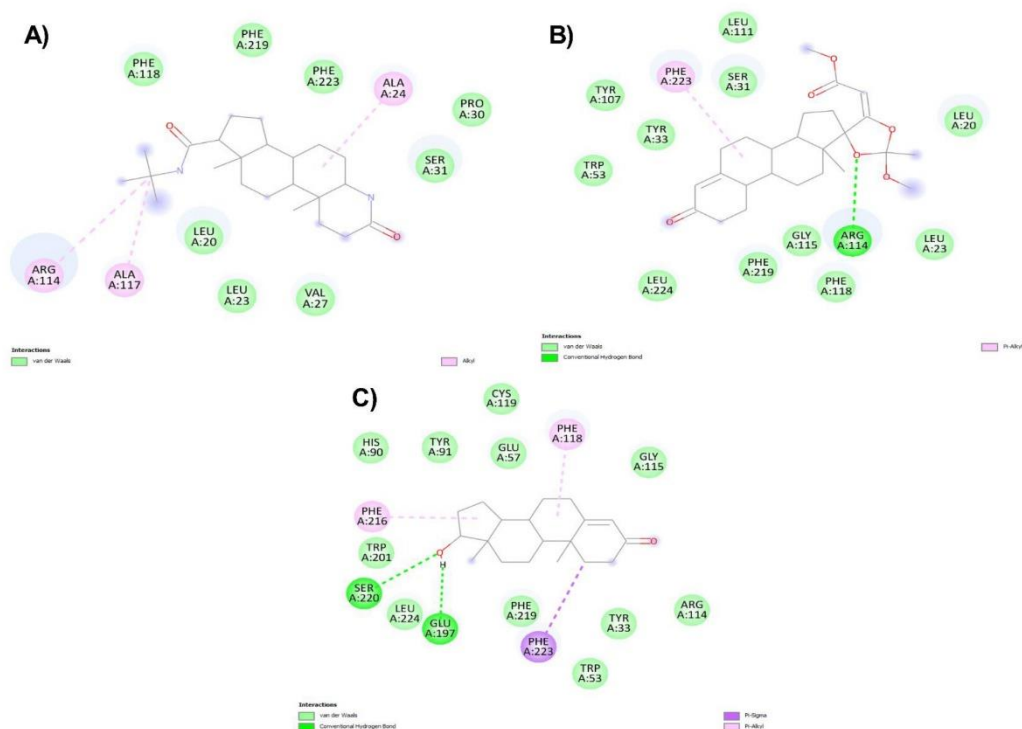


Figure S2. Protein-ligand interactions between 5αR2 and YK11 in 2D representations. A) Finasteride-5αR2 complex; B) YK11-5αR2 complex; C) Testosterone-5αR2 complex. Data obtained using the software AutoDockVina and Discovery Studio Visualizer.

6. DISCUSSÃO

Nosso estudo teve como objetivo avaliar os efeitos neuroquímicos da associação de doses anabólicas de YK11 ao exercício no hipocampo de ratos machos. O YK11, como um SARM esteroïdal, possui elevado potencial de atravessar a barreira hematoencefálica, o que nos levou a investigar suas respostas metabólicas especificamente no hipocampo. Além disso, a combinação com o exercício, prática frequentemente observada em usuários de substâncias ergogênicas, nos permitiu explorar os principais impactos dessa coadministração e verificar se os conhecidos efeitos neuroprotetores do exercício poderiam mitigar possíveis alterações neuroquímicas induzidas por doses anabólicas de YK11.

Esta dissertação é composta por dois artigos científicos que empregaram abordagens experimentais integradas, incluindo métodos *in silico*, *in vivo* e *ex vivo*, a fim de investigar a seletividade do YK11 e os potenciais efeitos adversos decorrentes de sua administração em uma dose anabólica, coadministrados em um protocolo de exercício. De forma abrangente, nossos achados revelam que, embora o exercício seja amplamente reconhecido por seus efeitos neuroprotetores no hipocampo (MAHALAKSHMI *et al.*, 2020; SIGARAN *et al.*, 2024), ele não foi capaz de atenuar grande parte das alterações neuroquímicas induzidas pelo YK11. Assim, nesta seção, discutiremos tais achados de forma integrada, abordando os resultados dos artigos científicos 1 e 2.

Nossas análises computacionais indicam que o YK11 possui menor afinidade pelo AR em comparação à DHT, mas ainda relevante, com interações no resíduo Arg752, essencial para a ativação do AR (LEVINE *et al.*, 2014; ORAFIDIYA *et al.*, 2018). Em relação à enzima 5 α RII, o YK11 mostrou maior afinidade que a finasterida, interagindo próximo ao resíduo Arg114, crucial para a ativação enzimática (KHANTAM *et al.*, 2021). A testosterona também interage com a 5 α RII, mas com menor proximidade aos resíduos de ativação, Arg114. Modelos de dinâmica molecular sugerem que o YK11 tem estabilidade superior à testosterona e comparável à finasterida no sítio ativo da 5 α RII. Esses achados reforçam a elevada afinidade do YK11 pelo AR, além de sugerirem seu potencial efeito inibitório sobre a 5 α RII, o que pode reduzir a conversão de testosterona endógena em DHT, resultando em níveis aumentados de testosterona livre e, consequentemente, amplificando os efeitos anabólicos-androgênicos (CHISLETT *et al.*, 2023). Adicionalmente, os próprios efeitos androgênicos do YK11 podem ser intensificados, uma vez que, além de sua ação direta no AR, SARMs, de maneira geral,

são conhecidos por não sofrer metabolização pela 5 α RII (BHASIN & JASUJA, 2009; CHRISTIANSEN *et al.*, 2020).

Para investigar os potenciais efeitos neuromodulatórios do YK11 no hipocampo, inicialmente realizamos simulações computacionais para avaliar sua distribuição em diversos sistemas biológicos. Utilizando modelagem farmacocinética baseada em fisiologia (PBPK), que integrou dados de distribuição metabólica de SARMs esteroidais, pesos reais dos animais, doses administradas e a via oral como método de administração, observamos uma alta compartimentalização do YK11 no cérebro e no tecido adiposo, coerente com sua natureza altamente lipossolúvel (KANNO *et al.*, 2013; 2020). Esse padrão de distribuição está alinhado com o descrito para outros SARMs de metabolismo semelhante, como o NEP28 (AKITA *et al.*, 2013). Além disso, uma vez que os SARMs, incluindo o YK11, são amplamente considerados seletivos para o músculo esquelético e com menor ação em outros tecidos (SOLOMON *et al.*, 2019; MACHAK *et al.*, 2020), realizamos dois experimentos subsequentes. Primeiramente, analisamos os níveis de AR no hipocampo, nosso tecido de interesse, para confirmar a possível distribuição de YK11 no SNC. Posteriormente, investigamos os efeitos da inibição da miostatina no músculo gastrocnêmio, buscando avaliar se, em doses anabólicas, haveria ativação androgênica em tecidos no hipocampo concomitante a ativação de marcadores de hipertrofia no tecido muscular.

Observamos uma redução significativa na razão citosol/núcleo de AR no hipocampo nos grupos tratados com YK11 e EXE+YK11, indicando a ação efetiva do YK11 nesse tecido. Essa interação facilita a formação de homodímeros do AR e sua translocação nuclear, promovendo efeitos anabólicos (KANNO *et al.*, 2011; MACHAK *et al.*, 2020). Simultaneamente, verificamos inibição da miostatina no músculo gastrocnêmio, amplificada pelo YK11 e pelo exercício. Já conhecida, a miostatina regula a síntese proteica em tecidos musculares, e sua inibição leva à hipertrofia (CARRARELLI *et al.*, 2015; LEGÅRD & PEDERSEN, 2019; SKRZYPCZAK *et al.*, 2021). Além de confirmar a eficácia do YK11 na inibição da miostatina, observamos maior captação no SNC, especificamente no hipocampo, um tecido altamente permeável a compostos androgênicos (SELAKOVIC *et al.*, 2019; JOKSIMOVIĆ *et al.*, 2019). Isso sugere uma ação dupla: anabólica no músculo e neuromoduladora no hipocampo. Logo, com base nesses resultados, investigamos parâmetros de modulação neuroquímica no hipocampo e possíveis interações com o exercício.

No grupo tratado com YK11, houve um aumento significativo na produção de espécies reativas e nos níveis de TBARS, indicando estresse oxidativo. O grupo EXE+YK11 mostrou proteção parcial, mas sem melhorias nas enzimas antioxidantes. Ambos os grupos apresentaram redução nas atividades das principais enzimas antioxidantes e nos níveis de Nrf2, enquanto a Hsp70, aumentou no grupo YK11 e foi restaurada no grupo EXE+YK11. Marcadores mitocondriais indicaram recuperação parcial no grupo EXE+YK11, mas o YK11 isolado causou uma redução significativa no número de células metabolicamente viáveis e nos níveis de proteína MnSOD/SOD2. Embora o exercício tenha revertido alguns efeitos negativos, o YK11 contribuiu para a diminuição da viabilidade celular. Estudos anteriores com EAAs sugerem que o declínio da atividade antioxidante está associado à diminuição do ciclo de renovação enzimática em células hipocâmpais, especialmente em doses elevadas (GOMES *et al.*, 2014; JOKSIMOVIĆ *et al.*, 2017; AGAHI *et al.*, 2022). Esses achados nos levaram a explorar marcadores de inflamação celular, buscando compreender os mecanismos de danos oxidativos induzidos por YK11.

A desregulação dos mecanismos inflamatórios no hipocampo, observada no grupo tratado com YK11, foi marcada pelo aumento de citocinas pró-inflamatórias, como IL-1 β , IL-6, e na citocinas anti-inflamatórias IL-10. A coadministração EXE+YK11 conseguiu reverter parcialmente esses danos, especialmente pela regulação da IL-6 e aumento da IL-10. Contudo, o tratamento isolado com YK11 não reduziu significativamente os níveis de IL-1 β e IL-6, além de aumentar os níveis de IL-10, sugerindo aumento da atividade inflamatória. Este perfil metabólico sugere uma ativação exacerbada das vias de sinalização inflamatória, com destaque para a via janus quinase (JAK) e o transdutor de sinal/ativador da transcrição (STAT), JAK-STAT, que é central em processos inflamatórios envolvendo interleucinas, especialmente a IL-6 (HU *et al.*, 2023; CUCU, 2022). No tratamento com YK11, a via JAK-STAT provavelmente media a inflamação, uma vez que não houve alterações significativas nos níveis de TNF- α , assim, possivelmente sem impactar a sinalização do receptor de fator de necrose tumoral 1 (TNFR1) (HU *et al.*, 2021). Esse perfil inflamatório, especialmente via IL-6, pode ativar vias apoptóticas associadas a inflamações crônicas (GEORGE *et al.*, 2021), comprometendo a integridade e função mitocondrial, além de desregular as proteínas envolvidas nos processos de fissão e fusão mitocondrial.

Embora o tratamento combinado EXE+YK11 tenha restaurado parcialmente a viabilidade celular e os níveis de MnSOD/SOD2, uma análise mais refinada indicou que

o efeito foi semelhante ao do YK11 isolado. Observou-se uma redução significativa nas proteínas das subunidades dos complexos mitocondriais, como NDUF8 (complexo I), CYTCB (complexo III) e ATP5A1 (complexo V), sugerindo comprometimento na funcionalidade da cadeia de transporte de elétrons (YU *et al.*, 2019; GUSDON *et al.*, 2017). Também houve diminuição nas proteínas de fusão mitocondrial, mitofusina 1 (Mfn1) e mitofusina 2 (Mfn2), essenciais para a integridade estrutural das mitocôndrias e a troca de componentes entre organelas (ALLEGRA *et al.*, 2019; LIANG & DANG, 2021). Essa redução prejudica o equilíbrio da dinâmica mitocondrial, afetando a capacidade adaptativa celular e diminuindo a eficiência metabólica (GREL *et al.*, 2023). Além disso, observou-se um aumento na proteína dinamina-1 (Drp1) fosforilada em Ser637, indicando intensificação da fissão mitocondrial e um ciclo de degradação mitocondrial por meio de mitofagia (YU *et al.*, 2019; GUSDON *et al.*, 2017; KO *et al.*, 2021).

As alterações no funcionamento mitocondrial ativam a cascata de apoptose, especialmente através das vias mitógenos p38 (p38 MAPK) e proteína quinase B (Akt). A p38 MAPK, uma quinase que responde a estresses celulares e inflamações, é crucial na regulação da apoptose e na resposta inflamatória (YANG *et al.*, 2014; YUE & LÓPEZ, 2020; BLOCK & RILEY, 2023). A avaliação da razão entre as formas total e fosforilada de p38 MAPK mostrou que sua ativação está associada ao início do processo apoptótico no hipocampo de animais tratados com YK11 e EXE+YK11, refletindo danos mitocondriais, mesmo sem alterações significativas em Akt. A Akt, sob condições normais, protege as células, inibindo a apoptose e promovendo a sobrevivência celular (YANG *et al.*, 2018; WANG *et al.*, 2021). Em inflamações menos severas, a Akt atua como regulador negativo da p38 MAPK, suprimindo sua atividade (KIM *et al.*, 2008; WIDENMAIER *et al.*, 2009; XU *et al.*, 2014). No entanto, em estresse crônico, a predominância da p38 MAPK sugere um desbalanceamento que favorece a apoptose, ativando proteínas pro-apoptóticas como Bax e diminuindo proteínas anti-apoptóticas como Bcl-2 (YUE & LÓPEZ, 2020; WHITAKER & COOK, 2021).

O tratamento com YK11 aumentou as razões Bax/Bcl-2, resultando em maior atividade da caspase-3, uma executora chave da apoptose (ZELLEROTH *et al.*, 2019). A caspase-3 cliva substratos celulares essenciais, promovendo a degradação celular e a apoptose (SHALINI *et al.*, 2015; ZELLEROTH *et al.*, 2019). Apesar do aumento de Bax em EXE+YK11, houve uma redução na caspase-3 clivada e restauração da Bcl-2, sugerindo efeitos neuroprotetores do exercício. Essa proteção parece estar relacionada à

preservação das defesas mitocondriais, evidenciada pelos níveis restaurados de MnSOD/SOD2, e na atividade de desidrogenases mitocondriais.

Diante da neurotoxicidade no hipocampo, investigamos respostas comportamentais relacionadas à consolidação de memória, focando na via pro-BDNF/BDNF/TrkB/CREB, essencial para a função cognitiva (BATHINA *et al.*, 2015; DAHLEH *et al.*, 2024). Os resultados mostraram que YK11 e EXE+YK11 reduziram a consolidação de memória no teste de esQUIVA inibitória, associados à diminuição de BDNF e TrkB, além da fosforilação reduzida de CREB. Perturbações nessas vias estão fortemente ligadas ao início de desordens neurodegenerativas, como a Doença de Alzheimer, onde a sinalização comprometida do BDNF/TrkB/CREB contribui para a acumulação de β -amiloide e diminuição da diferenciação neuronal (YIN *et al.*, 2018; LIU *et al.*, 2018), culminando em severas disfunções neurológicas a longo prazo.

Embora a combinação de EXE+YK11 tenha proporcionado alguma neuroproteção nas vias de apoptose e estresse oxidativo, a consolidação de memória permaneceu comprometida. Isso sugere que o YK11, mesmo quando associado ao EXE, não atenua os seus efeitos neurotóxicos relacionados ao abuso dessas substâncias. Isso é corroborado na literatura, onde o uso abusivo de substâncias sintéticas de testosterona está associado ao comprometimento da memória flexível de reconhecimento em ratos (WOOD & SERPA, 2020). Além disso, doses elevadas de boldenona e estanozolol, provocaram o aumento de comportamentos semelhantes à ansiedade e depressão nesses animais (BUENO *et al.*, 2020). Essas evidências sugerem que a neurotoxicidade induzida pelo YK11 compromete as vias neurotróficas de maneira semelhante a esses derivados androgênicos, possivelmente impactando negativamente a funcionalidade cognitiva a longo prazo. Isso levanta preocupações sobre o uso de SARMs, que, embora sugiram a possibilidade de maior seletividade e menos efeitos colaterais, podem acarretar consequências adversas significativas para a saúde cognitiva, de forma bastante análoga aos EAs.

7. CONCLUSÃO

Com base em nossos achados, podemos concluir que:

- I) O YK11 demonstrou afinidade pelo AR e potencial inibição da 5 α RII, sugerindo modulação significativa no eixo androgênico, com ação no hipocampo;

- II) No hipocampo, o YK11 aumentou o estresse oxidativo, com atenuação parcial pelos efeitos antioxidantes do exercício;
- III) No hipocampo, o YK11 reduziu as subunidades dos complexos I, III e V mitocondriais, comprometendo a eficiência energética;
- IV) No hipocampo, o YK11 desequilibrou os processos de fusão e fissão mitocondrial, prevalecendo a fissão;
- V) No hipocampo, o YK11 ativou vias apoptóticas, com proteção parcial oferecida pelo exercício;
- VI) No hipocampo, o YK11 elevou citocinas inflamatórias, com redução parcial pelo exercício, mas ainda acima dos níveis de controle;
- VII) No hipocampo, o YK11 comprometeu a consolidação da memória, plasticidade sináptica e vias neurobiológicas envolvidas na memória.

Coletivamente, nossos estudos demonstraram que o YK11, administrado em doses anabólicas, exibiu alta seletividade pelo AR no hipocampo, acompanhada por efeitos neurotóxicos acentuados. Esses efeitos foram evidenciados pelo aumento dos níveis de estresse oxidativo, levando à desregulação de proteínas mitocondriais e à ativação de vias intrínsecas de apoptose celular. Além disso, esses danos desencadearam processos inflamatórios que possivelmente comprometeram a consolidação da memória nos animais, além de provocar alterações na sinalização da plasticidade neural. Embora o exercício tenha desempenhado um papel neuroprotetor, seus benefícios foram limitados pela extensão dos danos induzidos pelo YK11.

8. PERSPECTIVAS FUTURAS

Com base no desenvolvimento desta dissertação e nos trabalhos publicados, verificou-se que a hipótese da menor seletividade dos SARMs em tecidos que não pertencem ao músculo esquelético e ósseo, especificamente o YK11, foi rejeitada. Nossas pesquisas demonstraram que o YK11 tem a capacidade de se distribuir no SNC especialmente em áreas com alta permeabilidade a substâncias androgênicas e anabólicas, neste caso, o hipocampo.

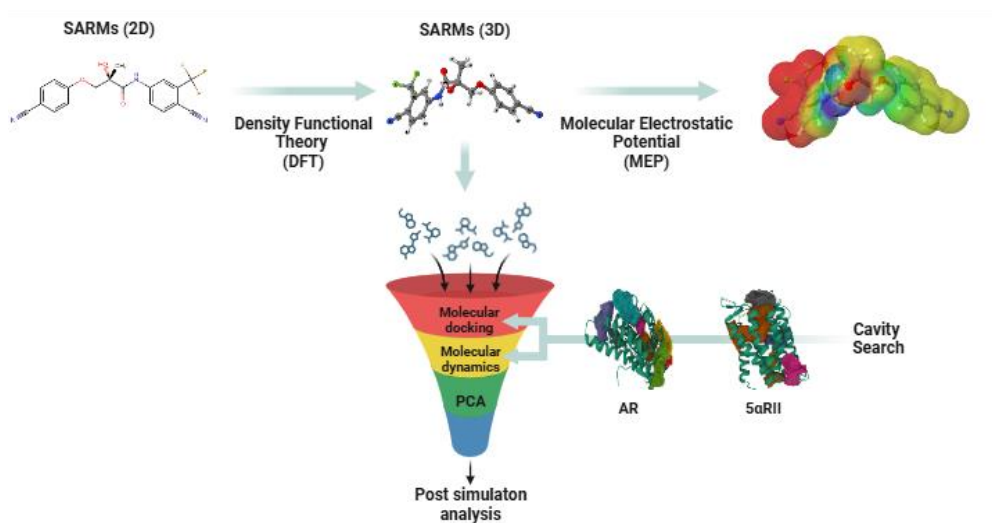
Com base nos dados obtidos, as perspectivas futuras englobam a continuidade dos experimentos voltados ao estudo de novas moléculas da classe dos SARMs em fase de testes pré-clínicos, como uma nova linha de pesquisa no Laboratório de Avaliações Farmacológicas e Toxicológicas Aplicadas às Moléculas Bioativas (LaftamBio Pampa).

Essa linha de pesquisa envolverá alunos de iniciação científica, mestrado e doutorado, abordando os seguintes temas:

- I) Avaliação computacional dos principais SARMs em fase de testes pré-clínicos, com foco em sua afinidade com diferentes espécies de receptores androgênicos (AR) e enzimas 5-alfa-redutase I e II (5 α RI/II);
- II) Investigação dos SARMs ligandrol e ostarina em ratos machos Wistar, analisando seus efeitos em outras regiões do SNC e demais tecidos periféricos;
- III) Comparação dos efeitos colaterais dos SARMs ligandrol e ostarina em relação a EAAs clássicos, como oximetolona, metandienona e metenolona, em ratos machos Wistar;
- IV) Comparação dos efeitos fisiológicos no metabolismo de ratos machos e fêmeas submetidos à administração de SARMs ligandrol e ostarina, em comparação com os EAAs clássicos supracitados.

Atualmente, durante a redação da dissertação, o item (I) já teve seus experimentos concluídos e está na fase final de elaboração do texto, conforme ilustrado no *graphical abstract*, que exemplifica como será apresentado este novo trabalho científico. Por outro lado, os itens (II) e (III) estão nas etapas iniciais de experimentação, em colaboração com membros do LaftamBio Pampa, cujos trabalhos de conclusão (TCC, dissertação e/ou tese) exploram alguns dos temas mencionados.

Graphical Abstract – Estudo computacional de SARMs



Fonte: Autor (2024).

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10. ANEXOS

Parecer de aprovação do Comitê de ética no Uso de Animais da UNIPAMPA: (CEUA)



CERTIDÃO

CERTIFICADO DE APROVAÇÃO DE PROTOCOLO PARA USO DE ANIMAIS EM PESQUISA

Número de protocolo da CEUA: 009/2022

Título: Avaliação do 19-norpregna-4,20-dieno-21-carboxílico, 17,20-[(1-metoxietilideno)bis(oxi)]-3-oxo-, metiléster (17 α , 20E) (YK11) associado ao exercício físico em parâmetros metabólicos, toxicológicos e inflamatórios em ratos Wistar

Data da aprovação: 25/05/2022

Período de vigência do projeto: 01/06/2024

Pesquisadores(a): Marina Prigol

Campus: Uruguiana

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Finalidade	() Ensino (X) Pesquisa
Espécie / Linhagem / Raça	Ratos Wistar
Nº de animais	72
Peso / Idade	200 a 250g / 2 meses
Sexo	Machos
Origem	Biotério Central UFSM ou Biopampa



Assinado eletronicamente por **ALESSANDRA SAYURI KIKUCHI TAMAJU SUKU NEIS**, Coordenador(a), em 01/06/2022, às 10:29, conforme horário oficial de Brasília, de acordo com as normativas legais aplicáveis.



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