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ENVOLVIMENTO DOS SISTEMAS SEROTONINÉRGICO E DOPAMINÉRGICO NA AÇÃO DO TIPO ANTIDEPRESSIVA DO 7-FLÚOR-1,3-DIFENILISOQUINOLINA-1-AMINO EM CAMUNDONGOS

DISSERTAÇÃO DE MESTRADO

Ana Paula Pesarico

Santa Maria, RS, Brasil
2014

ENVOLVIMENTO DOS SISTEMAS SEROTONINÉRGICO E DOPAMINÉRGICO NA AÇÃO DO TIPO ANTIDEPRESSIVA DO 7-FLÚOR-1,3-DIFENILISOQUINOLINA-1-AMINO EM CAMUNDONGOS

por

Ana Paula Pesarico

Dissertação apresentada ao Programa de Pós-Graduação em Ciências Biológicas, Área de Concentração em Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do grau de **Mestre em Bioquímica Toxicológica**

Orientadora: Profª Drª Cristina Wayne Nogueira

Santa Maria, RS, Brasil
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Universidade Federal de Santa Maria
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Elaborada por
Ana Paula Pesarico

como requisito parcial para obtenção do grau de
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COMISSÃO EXAMINADORA:

Cristina Wayne Nogueira, Drª
(Presidente, Orientadora)

Cristiane Luchese, Drª (UFPel)

Danieli Valnes Magni, Drª (URI)

Santa Maria, 11 de março de 2014

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"Se consegui enxergar longe é por que

estava apoiado sobre ombros de gigantes"

(Isaac Newton)

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica
Universidade Federal de Santa Maria, RS, Brasil

ENVOLVIMENTO DOS SISTEMAS SEROTONINÉRGICO E DOPAMINÉRGICO NA AÇÃO DO TIPO ANTIDEPRESSIVA DO 7-FLÚOR-1,3 DIFENILISOQUINOLINA-1-AMINO EM CAMUNDONGOS

AUTORA: ANA PAULA PESARICO
ORIENTADORA: CRISTINA WAYNE NOGUEIRA

Local e Data da Defesa: Santa Maria, 11 de março de 2014

A depressão é uma doença psiquiátrica associada com um impacto negativo na qualidade de vida. O sistema monoaminérgico parece estar envolvido nessa doença e na ação dos antidepressivos. Esse estudo teve como objetivo investigar o potencial do tipo antidepressivo do 7-flúor- 1,3 difenilisoquinolina-1-amino (FDPI) e o possível envolvimento do sistema monoaminérgico. Os resultados mostraram que o FDPI (1, 10 e 20 mg/kg, intragástrico (i.g.)) reduziu o tempo de imobilidade, aumentou o tempo de nado, mas não alterou o tempo de escalada dos camundongos durante o teste do nado forçado (TNF) modificado. Esses efeitos foram similares aos da paroxetina (8 mg/kg, intraperitoneal (i.p.)), um inibidor seletivo da recaptação de serotonina, o qual foi usado como controle positivo. Os pré-tratamentos com p-clorofenilalanina (*p*CPA, um inibidor da síntese de serotonina (5-HT), 100 mg/kg, i.p., uma vez por dia, por 4 dias consecutivos), N-{2-[4-(2-metoxifenil)-1-piperazinil]etil}-N-(2-piridinil) ciclohexanocarboxamida (WAY 100635, um antagonista dos receptores 5-HT_{1A}, 0,1 mg/kg, subcutâneo (s.c.)) e ondansetrona (um antagonista dos receptores 5-HT_3, 1 mg/kg, i.p.) conseguiram reverter o efeito do tipo antidepressivo do FDPI na dose de 1 mg/kg no TNF, o que não aconteceu com a ritanserina (um antagonista do receptores 5-$HT_{2A/2C}$, 1 mg/kg, i.p.). Antagonistas relacionados com o sistema dopaminérgico, como haloperidol (um antagonista do receptor D_2, 0,2 mg/kg, i.p.), e SCH23390 (um antagonista do receptor D_1, 0,05 mg/kg, s.c.) foram capazes de reverter o efeito do tipo antidepressivo do FDPI na dose de 1 mg/kg no TNF, o que não aconteceu com o sulpiride (um antagonista dos receptores D_2 e D_3, 50mg/kg, i.p.). O composto FDPI nas doses de 10 e 20 mg/kg inibiu a atividade da monoamino oxidase–B em córtex pré-frontal de camundongos. Estes resultados sugerem que o FDPI apresentou

uma ação do tipo antidepressiva no TNF em camundongos, possivelmente por um envolvimento do sistema monoaminérgico. Mais estudos se fazem necessários antes que se possa propor o FDPI como uma droga para o tratamento da depressão.

Palavras-Chave: Teste do Nado Forçado Modificado. Isoquinolina. Serotoninérgico. Dopaminérgico. Antidepressivo. Monoamino Oxidase.

ABSTRACT

Dissertation of Master's Degree
Federal University of Santa Maria, RS, Brazil

INVOLVEMENT OF SEROTONERGIC AND DOPAMINERGIC SYSTEMS IN THE ANTIDEPRESSANT-LIKE ACTION OF 7-FLUORO-1,3-DIPHENYLISOQUINOLINE IN MICE

AUTHOR: ANA PAULA PESARICO
ADVISOR: CRISTINA WAYNE NOGUEIRA

Place and Date of the defense: Santa Maria, March 11, 2014.

Depression is a psychiatric disorder associated with a negative impact on quality of life. Monoaminergic system has been involved in this disease and in the action of antidepressants. This study aimed to investigate the potential antidepressant-like of 7-fluoro-1,3-diphenylisoquinoline-1-amine (FDPI) and the possible involvement of monoaminergic system. Results showed that FDPI (1, 10 and 20 mg/kg, intragastric (i.g.)) reduced the immobility time, increased swimming time, but did not alter climbing time of mice in the modified forced swimming test (FST). These effects were similar to those of paroxetine (8 mg/kg, intraperitoneally (i.p.)), a selective serotonin reuptake inhibitor, which was used as positive control. Pretreatments with p-chlorophenylalanine (*p*CPA, an inhibitor of serotonin (5-HT) synthesis, 100 mg/kg, i.p., once a day for 4 consecutive days), N-[1]-N-(2-pyridinyl) cyclohexanecarboxamide (WAY 100635, a 5-HT_{1A} receptor antagonist, 0.1 mg/kg, subcutaneous injection (s.c.)) and ondansetron (a 5-HT_3 receptor antagonist, 1 mg/kg, i.p.) reversed the antidepressant-like effect of FDPI at the dose 1 mg/kg in FST, this did not occurs with ritanserin (a 5-$HT_{2A/2C}$ receptor antagonist, 1 mg/kg, i.p.). Antagonist related with dopaminergic system, as haloperidol (a D_2 receptor antagonist, 0.2 mg/kg, i.p.) and SCH23390 (a D_1 receptor antagonist, 0.05 mg/kg, s.c.) were able to reverse the antidepressant-like effect of FDPI at the dose 1 mg/kg in FST, this did not occurs with sulpiride (a D_2 and D_3 receptors antagonist, 50 mg/kg, i.p.). FDPI, at doses of 10 and 20 mg/kg, inhibited monoamine oxidase-B activity in prefrontal cortex of mice. These results suggest that FDPI produced an antidepressant-like action in the FST in mice, possibly by an involvement of the monoaminergic system. Additional studies are necessary in order to propose FDPI as a drug for depression treatment.

Keywords: Modified forced swim test; Isoquinoline; Serotonergic; Dopaminergic; Antidepressant-like; Monoamine Oxidase;

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INTRODUÇÃO

MANUSCRITO

LISTA DE ABREVIATURAS

FDPI - 7-Flúor-1,3-difenilisoquinolina-1-amino
5-HT - Serotonina
ISRS – Inibidores seletivos da recaptação de serotonina
MAO – Monoamino oxidase
SNC – Sistema nervoso central
TNF – Teste do nado forçado
NA – Noradrenalina
DA – Dopamina
IMAOs – Inibidores da monoamino oxidase
AMP cíclico – Adenosina monofosfato cíclico
OMS – Organização Mundial da Saúde
SERT – Proteina da recaptação da serotonina
5-HIAA – 5-Hidroxi-indol-acético
VMAT – Vesícula transportadora de monoaminas
PK – Proteína quinase
COMT – Catecol-O-metil-transferase

SUMÁRIO

1. INTRODUÇÃO

1.1 Depressão

A depressão é uma doença caracterizada por alterações psicológicas e comportamentais, incluindo anedonia (perda de interesse e prazer em atividades), sentimento de culpa e de desesperança, pensamentos suicidas, aumento ou diminuição do apetite e do sono; além de mudanças cognitivas, tal como a redução da capacidade de concentração (CRYAN et al., 2002; MORILAK e FRAZER, 2004).

O diagnóstico da doença baseia-se na observação clínica dos sintomas citados acima e enumerados na **Tabela 1**, que são altamente variáveis e muitas vezes contrastantes. Entretanto não é fácil de ser detectado entre os pacientes, o que permite o desenvolvimento e prolongamento da doença, comprometendo a qualidade de vida e aumentando o risco de doenças arteriais coronarianas e isquemia cerebral, além de morte do paciente (MURRAY e LOPEZ, 1996).

Para que o paciente seja diagnosticado com depressão maior, uma das manifestações mais graves da doença, é necessário que apresente no mínimo um critério dos dois primeiros mostrados na tabela 1 e apresentar o número necessário para completar um total de cinco entre os outros sintomas, sendo que esses sintomas devem ter duração mínima de duas semanas (American Psychiatric Association, 1994).

O transtorno depressivo acomete principalmente os idosos, no entanto está cada vez mais saliente entre adultos, jovens e crianças. Ademais, está associada com altas taxas de mortalidade e morbidade (NEMEROFF, 2007). Segundo estimativas da Organização mundial da Saúde (OMS) essa doença psiquiátrica afeta cerca de 121 milhões de pessoas mundialmente, o que representa 17% da população. A depressão tem sido estimada ser a segunda principal enfermidade ao final de 2020, perdendo apenas para as doenças cardiovasculares (MCKENNA et al., 2005). Contudo, tem sido associada à comorbidade de outras doenças psiquiátricas, bem como à ansiedade (NEMEROFF e OWENS, 2002).

Tabela 1. Sintomas da Depressão

1. Humor deprimido
2. Anedonia
3. Falta de esperança, desespero, sentimento de culpa ou desvalia
4. Perda de peso e apetite/ ganho de peso ou apetite
5. Agitação psicomotora/ letargia
6. Fadiga ou falta de energia
7. Pensamentos recorrentes de morte ou suicídio
8. Dificuldade de concentração
9. Insônia/ hiperinsônia

Fonte: Manual de Diagnóstico e Estatístico dos Distúrbios Mentais (American Psychiatric Association, 1994).

A neurobiologia dessa doença e o conhecimento preciso da sua etiologia ainda não são bem esclarecidos. Entretanto, é dito que a depressão pode originar-se tanto de fatores ambientais como de fatores genéticos. Os estudos na neurociência, principalmente na neurobiologia, estão avançando e o entendimento biológico entre o estado normal e patológico está sendo compreendido (FAVA e KENDLER, 2000; NESTLER et al., 2002).

1.2 Teoria monoaminérgica da depresssão

Várias pesquisas tem demonstrado que diferentes vias neuronais estão envolvidas na patofisiopatologia da depressão. A hipótese de maior relevância descoberta em 1967 (COPPEN, 1967), trata essa doença como um distúrbio no sistema monoaminérgico, o que é caracterizada por uma diminuição nos níveis cerebrais dos neurotransmissores serotonina (5-HT), noradrenalina (NA) e dopamina (DA). Diversos estudos sustentam a ideia da teoria das monoamias (MILLAN, 2004; SNOW et al., 2000). Nesse sentido, inibidores seletivos da recaptação de serotonina (ISRS), antidepressivos tricíclicos e inibidores da enzima monoamino oxidase (MAO), são alguns tipos de fármacos que apresentam efetividade no tratamento da depressão e estão relacionados ao contexto da hipótese do envolvimento do sistema monoaminérgico. Apesar dos ISRS serem medicamentos de primeira linha para o

tratamento da depressão, existe uma falha na ação desses medicamentos, uma vez que 1/3 dos pacientes não são responsivos ao protocolo de tratamento, além de possuírem inúmeras limitações, como lento início de ação e uma diversidade de efeitos coloterais (DUPUY et al., 2011).

1.2.1 Sistema Serotoninérgico

As alterações neuroquímicas e comportamentais da depressão são predominantemente causadas pelo sistema central serotoninérgico (GRIPPO et al., 2005; LI et al., 2009), sendo que a 5-HT, também dita 5-hidroxitriptamina, e seus receptores específicos são o foco de várias pesquisas em relação às doenças psiquiátricas.

No cérebro, a 5-HT é sintetizada a partir do aminoácido triptofano, através da enzima triptofano hidroxilase. Depois da biossíntese ela é estocada em vesículas (Figura 1). Essa ocorre exclusivamente em neurônios localizados nos núcleos da rafe, de onde partem projeções para todas as partes do sistema nervoso central (SNC) (WONG et al., 2005).

Esse neurotransmissor está envolvido no controle de uma diversidade de funções fisiológicas, bem como ingestão de alimentos, ciclo do sono e vigília, memória, comportamento sexual, locomoção, funções endócrinas e ainda no controle de nossas atividades emocionais e comportamentais (JACOBS e AZMITIA, 1992; LOWRY et al., 2008). O sistema de neurotransmissão serotoninérgica é constituído por 14 receptores, com sete classes distintas ($5\text{-}HT_{1\text{-}7}$) (HOYER et al., 2002; NICHOLS e NICHOLS, 2008).

Dos múltiplos receptores de serotonina presentes no cérebro, o receptor $5\text{-}HT_{1A}$ é um subtipo bem caracterizado que tem uma maior afinidade quando comparado a os outros subtipos (BOCKAERT et al., 2008; DAWSON e BROMIDGE, 2008). Esses receptores são acoplados a proteína G e são amplamente distribuídos nos neurônios pré-sinápticos de 5-HT do núcleo da rafe do mesencéfalo (auto-receptores) e nos neurônios pós-sinápticos nos terminais nervosos, principalmente em áreas córtico-límbicas (córtex frontal, septo, amígdala, hipocampo e hipotálamo) (CELADA et al., 2004; LUCKI et al., 1994). Os receptores localizados nos neurônios pré-sinápticos podem regular a liberação central de 5-HT. Ademais, tem sido mostrado que animais knockout para os receptores $5\text{-}HT_{1A}$ apresentaram uma redução no tempo de imobilidade no teste do nado forçado (TNF), um modelo animal comportamental para animais comumente usado na avaliação de compostos com ação do tipo

antidepressiva (HENSLER, 2003). Flesinoxan e ipsapirona são dois agonistas parciais dos receptores 5-HT_{1A}, que representam uma classe de antidepressivos. Estudos em relação a esses fármacos, mostram que uma longa exposição a eles podem causar uma disfunção nos receptores 5-HT_{1A}, além do mais, por serem parciais não alcançam uma resposta completa frente à depressão (NEMEROFF e OWENS, 2002).

Os receptores 5-HT_2 são amplamente distribuídos pelo cérebro, sendo principalmente encontrado no neocórtex, num padrão que sugere que sua ativação pode estar implicada na regulação dos transtornos do humor (CARR e LUCKI, 2011; CELADA et al., 2004). Diversas classes de antidepressivos apresentam interação com esses receptores, entre elas, os agentes tricíclicos, os quais expressam uma significante afinidade. A imipramina, um antidepressivo tricíclico, tem afinidade com esses receptores, porém seu efeito de inibir os transportadores de NA e 5-HT acaba por ofuscar o efeito sobre os receptores (BARBUI e HOTOPF, 2001).

Já foi relatado que no córtex pré-frontal de pacientes depressivos há um aumento na densidade dos receptores 5-HT_{2A} (SHELTON et al., 2009). Em outro estudo, com animais, foi observado que agonistas dos receptores 5-HT_{2C} tem um efeito do tipo antidepressivo no TNF e um antagonista seletivo desses receptores bloqueou esse efeito (CRYAN e LUCKI, 2000).

Os receptores 5-HT_3 são menos estudados que os demais receptores citados, mas já se sabe sobre o seu envolvimento na neurobiologia da depressão (BRUNING et al., 2011; SAVEGNAGO et al., 2007). Esses receptores são encontrados na área postrema, hipocampo e na amígdala (KILPATRICK et al., 1987). São responsáveis por uma liberação dos neurotransmissores, principalmente a DA, em uma rápida neurotransmissão excitatória (SUGITA et al., 1992).

Os receptores 5-HT_3 foram identificados como alvos de potentes drogas antidepressivas, como a fluoxetina, por bloquear os mesmos (RAJKUMAR e MAHESH, 2010). Estudos tem reportado que antagonistas seletivos para os receptores 5-HT_3 produzem um efeito do tipo antidepressivo (RAMAMOORTHY et al., 2008), além de potencializar o efeito anti-imobilidade dos ISRS (REDROBE e BOURIN, 1997).

As proteínas envolvidas na recaptação de 5-HT são um alvo para a procura de novos agentes terapêuticos. Essas proteínas tem a função de retirar a 5-HT da fenda sináptica e quando inibidas aumentam a concentração de 5-HT, aumentando o tônus do sistema serotoninérgico, o que consequentemente leva a uma diminuição dos sintomas depressivos (DUPUY et al., 2011; FAVA, 2003).

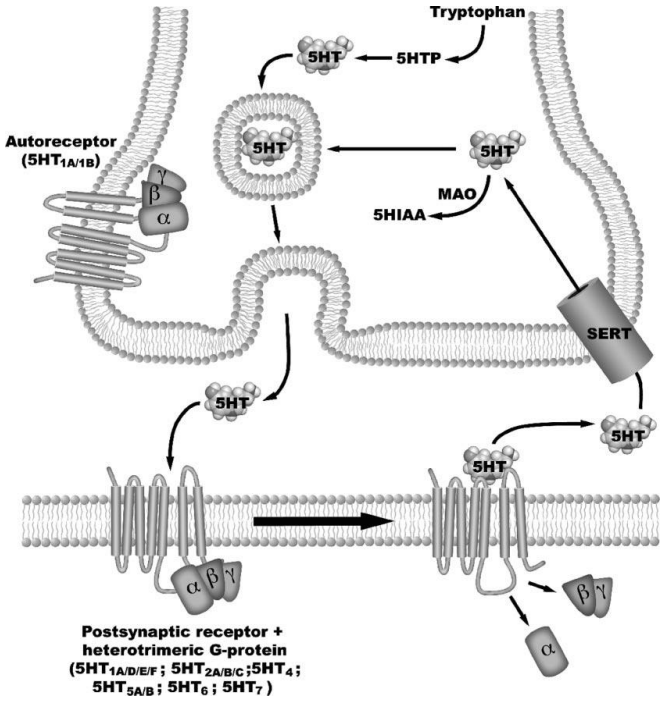

Figura 1: Modelo de uma sinapse serotoninérgica. Na sequência da sua biossíntese, a serotonina (5-HT) é estocada em vesículas. Quando um potencial de ação atinge a porção terminal, a despolarização da membrana leva ao influxo de cálcio e a fusão de vesículas com a membrana terminal. A 5-HT é liberada dentro do espaço sináptico, onde se difunde e ativa os receptores pós-sinápticos, iniciando as cascatas de sinalização dentro da célula. A 5-HT é retirada da sinapse por proteínas especializadas na membrana pré-sináptica, neste caso, a proteína da recaptação da 5-HT, chamada de SERT. A SERT capta a 5-HT livre para dentro do neurônio terminal, onde é reembalada em vesículas, para repetir o ciclo. A 5-HT que está livre no citoplasma e as que não estão armazenadas em vesículas são desaminadas pela monoamina oxidase na membrana mitocondrial para produzir o metabólito biologicamente inerte, ácido 5-hidroxi-indol-acético (5-HIAA) (Adaptado de Nichols e Nichols, 2007).

1.2.2 Sistema Dopaminérgico

Vários estudos demonstram o papel da neurotransmissão dopaminérgica na depressão maior. A DA é um neurotransmissor catecolaminérgico predominante no cérebro, responsável pelo controle de uma variedade de funções, incluindo emoção, atividade locomotora, concentração, regulação endócrina, prazer e consumo de comida (ALCARO et al., 2007). Um comprometimento dessas funções é uma das características envolvidas na depressão (COPPEN, 1967).

A DA é sintetizada nos neurônios pré-sinápticos do tronco cerebral, a partir de dois aminoácidos, fenilalanina e tirosina (Figura 2) (SZABO et al., 2004). Esses neurônios se

projetam por duas diferentes vias: a via meso-límbico-cortical (área tegmental ventral para o núcleo acúmbes) e a via que se projeta da substânica negra para o estriado dorsal.

Esse neurotransmissor exerce seu efeito nos neurônios pós-sinápticos, através da interação com 5 subtipos de receptores de dopamina, os quais estão divididos em dois grupos: família D_1 (abrangendo D_1 e D_5) e a família D_2 (abrangendo D_2, D_3 e D_4) (JARVIE e CARON, 1993; MISSALE et al., 1998). A diferença entre eles está relacionada ao seu mecanismo de ação, uma vez que a família D_1 estimula a enzima adenilato ciclase, aumentado os níveis de adenosina monofosfato cíclico (AMPc) no interior da célula, enquanto os receptores pertencentes a família D_2 inibem a adenilato ciclase e por consequência diminuem o AMPc (CLARK e WHITE, 1987; NIZNIK e VAN TOL, 1992).

Existe uma ampla distribuição de cada um dos receptores de dopamina no cérebro, sendo que os receptores D_1 e D_2 são expressos em vários sistemas neurais e os receptores D_3 e D_4 parecem estar em maior proporção no sistema límbico. Já os receptores D_5 tem sua distribuição mais restrita, além de serem encontrados geralmente em baixos níveis (SUNAHARA et al., 1991).

Evidências tem sugerido cada vez mais o envolvimento da DA na patofisiologia da depressão, uma vez que agonistas dos receptores dopaminérgicos aumentam o comportamento do tipo antidepressivo em modelos animais e que os antagonistas desses receptores revertem-no (JOCA et al., 2000; SCHULTE-HERBRUGGEN et al., 2012). Além disso, dados da literatura mostram que doenças psiquiátricas como a doença de Parkinson, esquizofrenia e depressão são caracterizadas por mudanças na sensibilidade e densidade dos receptores de DA, os quais estão presentes nas estruturas cerebrais dopaminérgicas (DI FORTI et al., 2007; DUNLOP e NEMEROFF, 2007).

1.2.3 Monoamino oxidase

A monoamino oxidase (MAO; EC 1.4.3.4) é uma enzima mitocondrial presente em quase todos os tecidos. No SNC ela é responsável pela desaminação de aminas, incluindo neurotransmissores monoaminérgicos (epinefrina, NA, DA e 5-HT). Foi demonstrado em 1968 a existência de 2 isoformas da MAO cerebral, tipo A (MAO-A) e tipo B (MAO-B). As quais se distinguem por diferentes afinidades em relação aos seus substratos e devido a sua sensibilidade (JOHNSTON, 1968). Sabe-se que a MAO-A possui alta afinidade pelos

substratos 5-HT, enquanto a MAO-B possui preferência para a feniletilamina, e ambas as enzimas atuam sobre a degradação de NA e DA (GAWESKA e FITZPATRICK, 2011; YAMADA e YASUHARA, 2004).

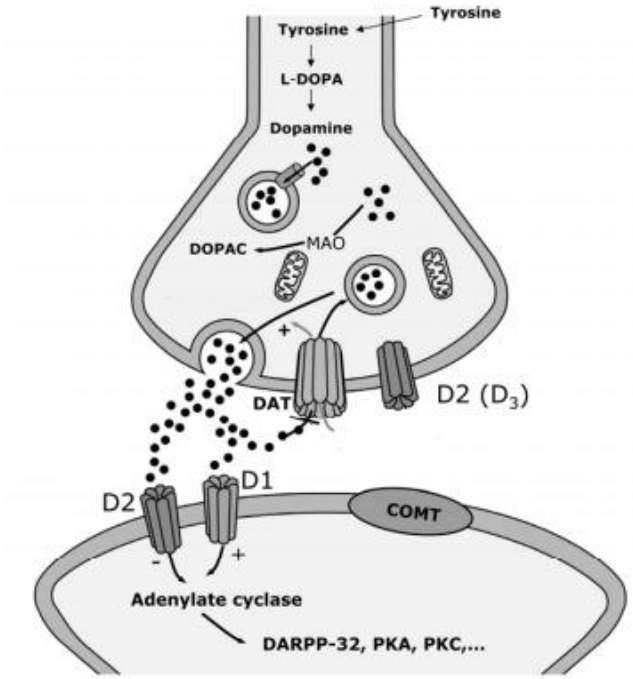

Figura 2. Esquema de uma sinapse dopaminérgica. Uma vez sintetizada a partir de L-tirosina e do L-DOPA, a dopamina (DA) é armazenada em vesículas sinápticas transportadoras de monoaminas, as quais internalizam as monoaminas e as protegem da degradação. A DA pode ser metabolizada em DOPAC pela monoamina-oxidase (MAO). Após a chegada de um impulso nervoso ao terminal do axônio, a DA é liberada por um mecanismo dependente de Ca^{2+}. A DA pode agir em receptores pré-sinápticos e pós-sinápticos de duas famílias diferentes (D1 e D2; D1 a D5) cuja ativação resulta em mudanças de AMPc e ainda ativação/inibição de várias proteínas quinases (PK). A concentração extracelular de DA é regulada por um proteína transportadora de membrana (DAT). A DA também pode ser metabolizada pela COMT (catecol - O- metil transferase). (Adaptado de Szabo et al., 2004).

Os inibidores da monoamina oxidase (IMAOs) foram incluídos aproximadamente 60 anos atrás, no tratamento da tuberculose (iproniazida), e junto a esse tratamento foi descoberto seu potencial em reduzir os sintomas depressivos. A partir disso, começaram-se os estudos e então foi determinada que o efeito antidepressivo da iproniazida fosse pelo bloqueio da MAO, o que leva a uma não remoção do grupamento amino dos neurotransmissores por essa enzima.

Para pacientes com o diagnóstico de transtorno de depressão maior, os IMAOs tem uma melhor eficácia quando comparada com outros antidepressivos, mas seus efeitos colaterais, em particular hipertensão tem levado os psiquiatras a não prescrever esses medicamentos. Uma pesquisa realizada em 1999 mostrou que 27% dos psiquiatras não

prescreveram os IMAOs nos últimos 3 anos e 12 % deles nunca prescreveram (BALON et al., 1999).

Atualmente, os IMAOs são usados para pacientes que não respondem as primeiras linhas de tratamento, bem como ISRS e os antidepressivos tricíclicos, sendo considerados resistentes ao tratamento. Pacientes depressivos que apresentam resistência ao tratamento é a maior causa de incapacidade e baixa produtividade, com altas taxas de hospitalização e ataques suicidas. Logo, é de grande importância ressaltar que pacientes que falham com outros antidepressivos podem responder aos IMAOs (MCGRATH et al., 1987; NOLEN et al., 1988).

1.3 Teste do nado forçado

Apesar dos avanços na compreensão da fisiopatologia da depressão e os esforços consideráveis para melhorar a eficácia dos tratamentos antidepressivos, cerca de 50 – 60% de todos os pacientes deprimidos permanecem parcialmente ou totalmente sem resposta à terapia com antidepressivos típicos, como os ISRS (FAVA, 2003; WARD e IRAZOQUI, 2010). Modelos de depressão em roedores tem sido desenvolvidos em um esforço para identificar novos antidepressivos e para promover uma melhor compreensão nos transtornos depressivos (WILLNER e MITCHELL, 2002). O mais importante e mais usado modelo em roedores para realização de um *screening* para novas drogas antidepressivas é o teste do nado forçado (TNF). O TNF, originalmente descrito por Porsolt et al. 1977, é um modelo onde ratos ou camundongos, são colocados em um cilindro com água de uma maneira a qual eles não possam escapar. Após um período de um comportamento de fuga, os roedores adotam uma postura imóvel, a qual prediz um comportamento do tipo depressivo. Tratamento agudo e por vezes crônico com novos compostos ou já conhecidos antidepressivos reduz o tempo de imobilidade e aumentam ou prolongam o comportamento de fuga, sem modificar a atividade locomotora (PORSOLT et al., 1977a; PORSOLT et al., 1977b).

Quando há uma modificação no TNF é possível observar dois parâmetros comportamentais ativos: comportamento de escalada, definido como movimentos para cima dirigido com as patas dianteiras, normalmente ao longo da parede do cilindro; comportamento de nadar, definido como o movimento (geralmente horizontal) em todo o cilindro que inclui passagem através de quadrantes do cilindro; e por fim a imobilidade, a qual é medida quando

nenhuma atividade adicional é observada, exceto o necessário para manter a cabeça do animal por cima da água (CAN et al., 2013; CRYAN et al., 2005; DETKE et al., 1995; TANAKA e TELEGDY, 2008). Esses comportamentos são alterados seletivamente por dois grupos diferentes de drogas antidepressivas.

O TNF modificado mostra que o comportamento de natação é aumentado em ISRS, enquanto que o comportamento de escalada aumenta quando as drogas antidepressivas tem efeitos seletivos sobre a inibição da recaptação de NA (DETKE et al., 1995; LUCKI, 1997).

1.4 Isoquinolinas

Derivados de isoquinolinas (Figura 3) são compostos orgânicos em que um anel de benzeno e um anel de piridina são fundidos através de carbono (DEITRICH e ERWIN, 1980; ROMMELSPACHER et al., 1991). Esses compostos podem ser encontrados em uma variedade de alimentos, bem como cacau, banana e leite (NIWA et al., 1989), além de estarem presentes no cérebro de mamíferos, como por exemplo o 1,2,3,4-tetrahidroisoquinolina (NIWA et al., 1989; YAMAKAWA e OHTA, 1997) e o 1-metil-1,2,3,4–tetrahidroisoquinolina (KOTAKE et al., 1995).

A tetrahidroisoquinolina, um composto endógeno, apresenta diversas funções biológicas, incluindo neuroproteção e um efeito do tipo antidepressivo (ANTKIEWICZ-MICHALUK et al., 2006; ANTKIEWICZ-MICHALUK et al., 2013; PATSENKA e ANTKIEWICZ-MICHALUK, 2004). Tem se demonstrado que uma série de compostos derivados da tetrahidroisoquinolina inibi a atividade das isoformas da monoamino oxidase e também aumenta os níveis dos neurotransmissores, através da inibição da atividade da enzima tirosina hidroxilase (PATSENKA e ANTKIEWICZ-MICHALUK, 2004).

Inúmeras pesquisas tem demonstrado o papel de outros compostos derivados de isoquinolinas, naturais ou sintéticos, em diversas patologias, incluindo o transtorno de humor. A Berberina, por exemplo, é um alcaloide isoquinolínico encontrado em várias espécies de plantas e que apresenta diferentes propriedades farmacológicas, incluindo anti-inflamatória (KUPELI et al., 2002), cardioprotetora (ZHENG et al., 2003), antitumoral (KETTMANN et al., 2004), antioxidante (RACKOVA et al., 2004) e neuroprotetora (MA et al., 1999). Dentre os efeitos neuroprotetores, inclui-se o efeito antidepressivo e ansiolítico. O efeito do tipo antidepressivo foi apresentado em dois modelos animais, além disso, o mecanismo de ação foi

demonstrado ser através da modulação no aumento dos níveis de 5-HT, NA e DA em hipocampo e córtex frontal de roedores (KULKARNI e DHIR, 2008; PENG et al., 2007). Além disso, a berberina tem um efeito inibitório na atividade da enzima monoamino oxidase cerebral (KONG et al., 2001).

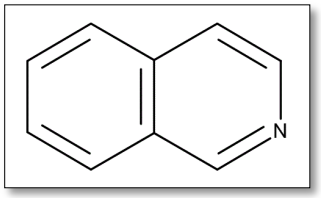

Figura 3: Estrutura química da Isoquinolina

Além das isoquinolinas já ressaltadas, existem outros alcaloides isoquinolínicos, com potentes atividades biológicas, bem como antimicrobiana (GALAN et al., 2013), antinociceptiva (KUPELI et al., 2002), citotóxica (WRIGHT et al., 2000), *scavenger* do radical hidroxila (JANG et al., 2009), propriedades antipsicóticas, ansiolíticas e antidepressivas, com um perfil modulador dos receptores serotoninérgicos e dopaminérgicos ($5\text{-}HT_{1A}$, $5\text{-}HT_{2A}$, $5\text{-}HT_{7}$, D_2 e D_3) (ZAJDEL et al., 2013; ZAJDEL et al., 2012).

Nesse contexto, o composto 7-flúor-1,3-difenilisoquinolina-1-amino (FDPI), representado na Figura 4, um novo composto sintético derivado de isoquinolina, demonstrou ser um potente agente antioxidante e um inibidor não seletivo da MAO em baixas concentrações *in vitro*. Além disso, demonstrou uma ação do tipo antidepressiva, não apresentando nenhuma toxicidade aguda cerebral, hepática e renal (MANTOVANI et al., 2014).

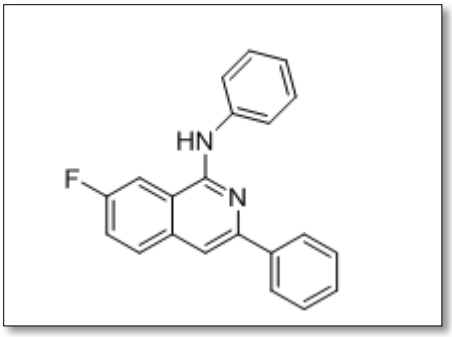

Figura 4: Estrutura química do FDPI

Diante do exposto, considerando a necessidade do desenvolvimento de novos tratamentos para a depressão, o envolvimento do FDPI com o sistema monoaminérgico e o papel deste na depressão, o FDPI torna-se um interessante candidato à agente terapêutico da depressão.

2. OBJETIVOS

2.1 Objetivo geral

O objetivo geral do presente trabalho foi avaliar o efeito do tipo antidepressivo do composto FDPI em camundongos no TNF modificado, bem como investigar os mecanismos pelos quais o FDPI age.

2.2 Objetivos específicos

Considerando os aspectos mencionados, os objetivos específicos deste estudo compreendem:

- Avaliar a possível ação do tipo antidepressiva do FDPI no TNF modificado em camundongos;
- Verificar se o sistema serotoninérgico (receptores 5-HT_{1A}, 5-$HT_{2A/2C}$ e 5-HT_3) e dopaminérgico (receptores D_1, D_2 e D_3) estão envolvidos na ação do tipo antidepressiva do FDPI.
- Investigar o possível envolvimento das isoformas da monoamino oxidase (MAO-A e B) na ação antidepressiva do FDPI;
- Avaliar o possível efeito do FDPI na captação de serotonina;

3. RESULTADOS

Os resultados que fazem parte dessa dissertação estão apresentados na forma de um manuscrito. Os itens Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas do manuscrito estão dispostos de acordo com a recomendação do periódico científico no qual o mesmo foi submetido.

3.1 Manuscrito

The antidepressant-like effect of 7-fluoro-1,3-diphenylisoquinoline-1-amine in the mouse forced swimming test is mediated by serotonergic and dopaminergic systems

Ana Paula Pesarico[a], Tuane Bazanella Sampaio[a], Anderson Mantovani[a], Gilson Zeni[a], Cristina Wayne Nogueira[a *]

[a]Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, Rio Grande do Sul, Brasil

*Correspondence should be sent to:

Cristina Wayne Nogueira

Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil.

Phone: 55-55-3220-9611

FAX: 55-55-3220-8978

E-mail: criswn@quimica.ufsm.br

Abstract

The aim of the present study was to specify the role of monoaminergic systems in the mechanism of 7-fluoro-1,3-diphenylisoquinoline-1-amine (FDPI) antidepressant-like action in mice. The antidepressant-like effect of FDPI was characterized in the mouse modified forced swimming test (FST) and the possible mechanism of action was investigated by using serotonergic and dopaminergic antagonists. Monoamine oxidase (MAO) activity and [^{3}H] serotonine (5-HT) uptake were determined in prefrontal cortices of mice. The results showed that FDPI (1, 10 and 20 mg/kg, i.g.) reduced the immobility time and increased the swimming time but did not alter climbing time in the modified FST. These effects were similar to those of paroxetine (8 mg/kg, i.p.). Pretreatments with *p*-chlorophenylalanine (100 mg/kg, i.p.), WAY 100635 (0.1 mg/kg, s.c.), ondansetron (1 mg/kg, i.p.), haloperidol (0.2 mg/kg, i.p.) and SCH23390 (0.05 mg/kg, s.c.), but not with ritanserin (1 mg/kg, i.p.) and sulpiride (50 mg/kg, i.p.), were effective to block the antidepressant-like effect of FDPI at a dose of 1 mg/kg in the FST. FDPI at doses of 1, 10 and 20 mg/kg did not change synaptosomal [^{3}H] 5-HT uptake. FDPI at doses of 10 and 20 mg/kg inhibited MAO-B activity. These results suggest that FDPI produced an antidepressant-like action in the modified FST. The results indicate that the antidepressant-like effect of FDPI is mediated by serotonergic and dopaminergic systems.

Keywords: Modified forced swim test; FDPI; Serotonergic; Dopaminergic; Antidepressant-like; Monoamine Oxidase;

Introduction

Depression is one of the most prevalent neuropsychiatric disorders in today's society characterized by modifications of mood and emotions. It affects approaching 15–25% of population [1] and studies predict that in 2020, it will be the second largest global burden of disease, illustrating the severity and impact of the disorder [2,3].

Studies have demonstrated the involvement of numerous neural pathways in the pathophysiology of depression [4,5]. The monoaminergic system is one of the most important targets in this disorder [6]. Furthermore, numerous antidepressant compounds are now available, which probably act via different mechanisms, including the serotonergic, noradrenergic and dopaminergic systems [7].

Among the classical pharmacological treatments for depression currently available, three main classes can be cited: tricyclic antidepressants (TCAs), selective serotonin (5-HT) reuptake inhibitors (SSRIs) and monoamine oxidase inhibitors (MAOIs). However, it often takes more than 5–8 weeks until the patients respond to the treatment, beyond present adverse effects [8]. Thus, pursuing new pharmacotherapy, with elevated efficacy and fewer adverse side-effects, is an utmost clinical need.

Besides the well known involvement of monoamines, currently, large scales of studies about the antidepressant mechanisms start to focus on the monoamine receptors. Different serotonergic receptors (5-HT_{1A}, 5-$HT_{1B/1D}$ and 5-HT_{2C}) have been implicated to mediate antidepressant responses in animal behavioral models, such as forced swimming test (FST) [9,10]. In addition, dopaminergic receptor antagonists reverse the antidepressant-like effect that has been shown by dopamine receptor agonists [11,12]. Pharmacological data suggest that serotonergic and dopaminergic systems are involved in the mechanisms by which drugs with antidepressant-like effect act [13,14].

The forced swimming test (FST), is the most widely used pharmacological model for assessing antidepressant efficacy [15,16] and is based on the adoption of a passive response in a stress situation and is suggested to have greater sensitivity [17]. The modification in the FST allows detecting active behaviors as either swimming, which is sensitive to serotonergic compounds such as the selective serotonin reuptake inhibitors (SSRIs), or climbing, which is sensitive to noradrenalin (NE)-selective uptake inhibitors [18,19].

Substituted isoquinolines represent a class of several natural and synthetic compounds [20,21]. The 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) is an endogenous substance, which demonstrates antidepressant-like activity in the FST [22]. 1MeTIQ inhibits both monoamine oxidase A (MAO-A) and B (MAO-B) activities and increases monoamine neurotransmitter levels in the brain [23]. Berberine, a yellow plant isoquinoline alkaloid, has multiple neuropharmacological properties, such as antidepressant and anxiolytic effects [24]. 7-Fluoro-1,3-diphenylisoquinoline-1-amine (FDPI), a synthetic isoquinoline represented in Figure 1, has been reported as an compound with antidepressant-like action in mice. This compound shows be a nonselective MAO inhibitor, besides of antioxidant properties *in vitro* [21].

In view of the above considerations, the antidepressant-like effect of FDPI was investigated in the mouse modified FST. The hypothesis that serotonergic and dopaminergic systems are involved in the antidepressant-like action of FDPI in the FST was evaluated.

Materials and methods

Animals

Behavioral experiments were conducted using male Swiss mice (25–35 g). Animals were maintained at 22–25 ^{0}C with free access to water and food, under a 12:12 h light/ dark

cycle, with lights on at 7:00 a.m. All manipulations were carried out between 08.00 a.m. and 04.00 p.m. All experiments were performed on separate groups of animals and each animal was used only once in each test. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, of the Federal University of Santa Maria, Brazil. All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

Chemicals

FDPI (Figure 1) was prepared and characterized in our laboratory by the method previously described [21]. Analysis of the 1HNMR and 13CNMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of studied compound (99.9 %) was determined by gas chromatography–mass spectrometry. All other chemicals were of analytical grade and obtained from standard commercial suppliers. To behavioral assays, all drugs were dissolved in saline except FDPI that was dissolved in canola oil. Mice received all drugs in a constant volume of 10 ml/kg body weight.

Behavioral tests

Forced swimming test (FST)

The modified mouse FST was conducted as described by [18] and Porsolt et al. [25], with some modifications [26,27]. Briefly, mice were individually forced to swim in an open cylindrical container (15 cm in diameter and 40 cm in height), containing 30 cm of water at 25±1 ^{0}C. In the test, the time of climbing, swimming and immobility was measured during a

6-min period. Climbing behavior consisted of upward directed movements of the forepaws along the side of the swim chamber. Swimming behavior was defined as movement (usually horizontal) throughout the swim chamber, which also included crossing into another quadrant. Immobility was assigned when no additional activity was observed other than that required to keep the mice head above the water.

The FST, as originally described by Porsolt et al. [17,25], was carried out for studying the involvement of serotonergic and dopaminergic systems in the antidepressant-like effect of compound. In this test, mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25 ± 1 ^{0}C. The total duration of immobility was recorded during the 6-min period. A decrease in the duration of immobility is indicative of an antidepressant-like effect.

Effect of FDPI in the modified mouse FST

In this test, 30 min after the intragastric (i.g.) administration of FDPI (1, 10 and 20 mg/kg) or canola oil (vehicle), the animals were placed in the apparatus for 6 min and the behaviors were monitored. Pretreatment time of 30 min for administration of FDPI was based on a previously published report [21]. Paroxetine (8 mg/kg, intraperitoneally (i.p.), a selective serotonin reuptake inhibitor) [28], administered 45 min before the modified forced swimming test, was used as positive control.

The role of the serotonergic system in the antidepressant-like effect of FDPI in the FST

To address the role of the serotonergic system in the antidepressant-like effect of FDPI in the forced swimming test, distinct groups of animals were treated with different classes of drugs. Mice were pretreated with ondansetron, a selective 5-HT_3 receptor antagonist (1

mg/kg, i.p.) [29], ritanserin, a non-selective 5-$HT_{2A/2C}$ receptor antagonist (4 mg/kg, i.p.) [10] or [10]N-[1]-N-(2-pyridinyl) cyclohexanecarboxamide (WAY100635), a selective 5-HT_{1A} receptor antagonist (0.1 mg/kg, subcutaneous injection (s.c.)) [29], 15 min before of FDPI (1 mg/kg, p.o.) or canola oil (10 ml/kg, i.g.) and 30 min after FDPI the FST was carried out.

p-Chlorophenylalanine (*p*CPA) is known to reduce the concentration of brain 5-HT by inhibiting its biosynthesis [30]. In the present experiments, mice were injected i.p. either with saline (control group) or with *p*CPA. *p*CPA was administered at a dose of 100 mg/kg once daily for 4 consecutive days [31]. On the fifth day (24 h after the last *p*CPA administration), mice received canola oil (10 ml/kg, i.g.) or FDPI (1 mg/kg, i.g.) 30 min before the test.

The role of the dopaminergic system in the antidepressant-like effect of FDPI in the FST

To study the possible contribution of the dopaminergic system in the antidepressant-like action of FDPI, separate groups of animals were pretreated with haloperidol (0.2 mg/kg, i.p., a dopamine D_2 receptor antagonist), SCH23390 (0.05 mg/kg, s.c.), a dopamine D_1 receptor antagonist), sulpiride (50 mg/kg, i.p., a dopamine D_2 and D_3 receptor antagonist) or saline (10 mg/kg, i.p.). After 15 min, they received FDPI (1 mg/kg, i.g.) or canola oil (10 ml/kg, i.g.) and 30 min later, mice were tested in the FST [32,33].

Spontaneous locomotor activity

With the purpose of excluding sedative or motor abnormality, the spontaneous locomotor activity was tested in mice. The animals were exposed to the chamber before testing, and activity was monitored under light and sound-attenuated conditions. Testing took place in a clear acrylic chamber (500 × 480 × 500 mm) equipped with 16 infrared sensors for

the automatic recording of horizontal activity (Model EP149, Insight Instruments Ltda, São Paulo, BR). Each animal initially was placed in the center of the testing chamber and allowed to freely move while being tracked by an automated tracking system. The data (rearing, distance, velocity and crossings) were collected and recorded during 4 min.

Ex vivo assays

Effect of FDPI on MAO-A and MAO-B activities

To test the hypothesis that antidepressant-like effect of FDPI is mediated through an inhibition of MAO-A or MAO-B activity, mice were pretreated with FDPI (1, 10 and 20 mg/kg, i.g.) or vehicle (canola oil, 10 ml/kg, i.g.). After 30 min, animals were killed by cervical dislocation and prefrontal cortex was removed and used for determining MAO activity.

Mitochondria preparation

A mitochondrial preparation of prefrontal cortex was used for MAO assay as previously described by Soto-Otero et al. [34]. The prefrontal cortex was immediately removed and washed in ice-cold isolation medium (pH 7.4, Na_2PO_4/KH_2PO_4 isotonized with sucrose). Mitochondria from cortex were then obtained by differential centrifugation. Briefly, after removing blood vessels and pial membranes, cerebral cortices were manually homogenized with four volumes (w/v) of the isolation medium. Then, the homogenate was centrifuged at 900 x g at 4 ^{0}C for 5 min. The supernatant was centrifuged at 12.500 x g for 15 min. The mitochondria pellet was then washed once with isolation medium and recentrifuged

under the same conditions. Finally, the mitochondrial pellet was reconstituted in a buffer solution (Na_2PO_4/KH_2PO_4 isotonized with KCl, pH 7.4) and stored in aliquots.

Enzyme assay

MAO activity was determined as described by Krajl [35] with some modifications of Matsumoto et al. [36]. Aliquots of samples were incubated at 37 ^{0}C for 5 min in a medium containing buffer solution (Na_2PO_4/ KH_2PO_4 isotonized with KCl, pH 7.4) and specific inhibitors, selegiline (a MAO-B inhibitor, 250 nM) or clorgiline (a MAO-A inhibitor, 250 nM), at a final volume of 600 µl. Then kynuramine dihydrobromide (final concentration 90 mM to MAO-A assay and 60 mM to MAO-B assay) was added to the reaction mixture as substrate. Samples were then incubated at 37 ^{0}C for 30 min. After incubation, the reaction was terminated by adding of 10% of trichloroacetic acid (TCA). After cooling and centrifugation at 16000 x g for 5 min, an aliquot of supernatant was added to 1M NaOH. The fluorescence intensity was detected spectrofluorimetrically with excitation at 315 nm and emission at 380 nm. The concentration of 4-hydroxyquinoline was estimated from a corresponding standard fluorescence curve of 4-hydroxyquinoline. MAO-A and MAO-B activities were expressed as nmol of 4-hydroxyquinoline formed/mg protein/min.

Effect of FDPI on [^{3}H]5-HT uptake

To test the hypothesis that the antidepressant-like effect of FDPI is mediated through an inhibition of 5-HT uptake, mice were pretreated with FDPI (1, 10 and 20 mg/kg, i.g.) or vehicle (canola oil, i.g.), after 30 min animals were killed by cervical dislocation and the prefrontal cortex was removed.

Preparation of crude synaptosomes

Crude synaptosomes were obtained as described by Gray and Whittaker [37] with some modifications. The prefrontal cortex was placed into ice-cold sucrose solution (0.32 M, pH 7.4), cut into small pieces and homogenized using a glass Potter-Elvehjem tube with a Teflon pestle (10 up and down strokes). The homogenate solution was centrifuged at 1000 x g at 4 ^{0}C for 10 min in a refrigerated centrifuge. The pellet was discarded and the supernatant was subsequently centrifuged at 12000 x g at 4 ^{0}C for 20 min. The final pellet of this centrifugation was suspended in ten volumes of ice-cold sucrose solution (0.32 M, pH 7.4) and then used as a crude synaptosome preparation in the [^{3}H]5-HT uptake assay.

[^{3}H]5-HT uptake assay

[^{3}H] 5-HT uptake into synaptosomes was carried out as described by Yura et al. [38] with some modifications. The synaptosomal suspension (125 µg of protein) was pre-incubated at 37 ^{0}C for 10 min in physiological salt solution (pH 7.4, adjusted with phosphoric acid 1%) of the following composition: 108 mM NaCl, 1 mM KCl, 27 mM $NaHCO_3$, 1.1 mM NaH_2PO_4, 0.1 mM pargyline, 0.1 mM ascorbic acid, 0.1 mM EDTA, 10 mM glucose, and, 5 mM $CaCl_2$. After preincubation, [^{3}H] 5-HT uptake was initiated by the addition of 7 nM [^{3}H] 5-HT (specific activity: 23 Ci/mmol) and 43 nM non-radioactive 5-HT (total 5-HT: 50 nM, final concentration). Synaptosomes were incubated for a further 2 min at 37 ^{0}C. 5-HT uptake was stopped by the immediate placement of assay tubes into ice, followed by centrifugation at 2000 x g at 4 ^{0}C for 5 min. Final pellets were washed with cold incubation buffer. Radioactivity present in pellet was measured in a scintillation counter. The non-specific activity was obtained in the presence of 100 mM paroxetine at 4 ^{0}C. Specific [^{3}H] 5-HT

uptake was indirectly estimated by subtracting the non-specific uptake from the total uptake determined at 37 ^{0}C. Results were expressed as *f* mol of 5-HT uptake/mg of protein/min.

Protein determination

The protein concentration was measured by the method of Bradford [39] using bovine serum albumin as a standard.

Statistical analysis

Different doses of FDPI (1, 10 and 20 mg/kg) in the modified FST and spontaneous locomotor activity were analyzed by one-way analysis of variance (ANOVA), followed by the Newman‑Keuls test. In behavioral tests, comparisons between paroxetine and control group were performed by the Student *t* test.

Ex-vivo data (MAO activity and [^{3}H]5-HT uptake) were analyzed by one-way analysis of variance followed by the Newman‑Keuls test.

The two-way ANOVA of variance followed by the Newman‑Keuls test was used to assess the effects of FDPI x antagonists in the FST. Main effects are presented only when the first order interaction was non-significant. Descriptive statistics data were expressed as the mean(s) $\pm$ S.E.M. Probability values less than 0.05 ($p < 0.05$) were considered as statically significant.

Results

Behavioral tests

Effect of FDPI in the mouse modified FST

The effects of different doses of FDPI or paroxetine on the climbing, swimming and immobility time in the modified mouse FST are shown in Figure 2.

One-way ANOVA showed that there was no effect of FDPI on climbing time. FDPI at all doses tested increased swimming time [$F_{(3,22)}$=6.998, p=0.0018] and decreased immobility time [$F_{(3,23)}$=5.935, p=0.0038] (Figure 2).

The Student t test revealed that paroxetine increased swimming time (p=0.0001) and decreased immobility time (p=0.0009) in the mouse FST when compared to the control group. Similar to FDPI, paroxetine did not increase significantly the climbing.

The role of the serotonergic system in the antidepressant-like effect of FDPI in the FST

Figure 3A shows that pretreatment of mice with ondansetron was effective in preventing the antidepressant-like action of FDPI in the FST. The two-way ANOVA of immobility time demonstrated a significant difference for FDPI x ondasentron interaction ($F_{(1,28)}$= 14.817, p=0.00063).

Figure 3B shows that pretreatment of mice with ritanserin was not effective in abolishing the antidepressant-like action of FDPI in the FST. The two-way ANOVA of immobility time demonstrated main effect of FDPI ($F_{(1,28)}$= 20.028, p=0.00015).

Figure 3C shows that pretreatment of mice with WAY100635 prevented the antidepressant-like action caused by FDPI in the FST. The two-way ANOVA of immobility time demonstrated a significant difference for FDPI x WAY100635 interaction ($F_{(1,22)}$= 15.672, p=0.00067).

Figure 3D demonstrates that pretreatment of mice with *p*CPA was effective in preventing the antidepressant-like action of FDPI in the FST. The two-way ANOVA of immobility time demonstrated a significant difference for FDPI x *p*CPA interaction ($F_{(1,26)}$= 5.8951, *p*=0.02241).

The role of the dopaminergic system in the antidepressant-like effect of FDPI in the FST

Figure 4 shows that pretreatment of mice with haloperidol blocked the effect of FDPI in the FST. The two-way ANOVA of immobility time demonstrated a significant difference for FDPI x haloperidol interaction ($F_{(1,26)}$= 8.0501, *p*=0.00870).

Pretreatment of mice with SCH23390 prevented the antidepressant-like effect caused by FDPI in the FST. The two-way ANOVA of immobility time revealed a significant difference for FDPI x SCH23390 interaction ($F_{(1,26)}$= 11.896, *p*=0.00193).

Pretreatment with sulpiride did not reverse the antidepressant-like effect of FDPI in the FST. The two-way ANOVA of immobility time revealed a main effect of FDPI ($F_{(1,22)}$= 13.333, *p*=0.00141).

Spontaneous locomotor activity

One-way ANOVA of spontaneous locomotor activity parameters (crossings, distance, rearings and velocity) did not demonstrate effect of FDPI at all doses tested (Table 1). The Student *t* test revealed that paroxetine increased crossing (*p*=0.0295) and distance (*p*=0.0395), but did not alter rearing (*p*=0.6751) and velocity (*p*=0.0833).

Ritanserin decreased all parameters in the spontaneous locomotor activity of mice when compared to the control group. Two-way ANOVA showed a significant main-effect of

ritanserin on the number of crossings [$F_{(1,24)}$=34.641, p=0.00000], number of rearings [$F_{(1,24)}$=19.875, p=0.00016], distance [$F_{(1,24)}$=43.592, p=0.00000] and velocity [$F_{(1,24)}$=9.4489, p=0.00520] (Table 1).

Haloperidol and SCH23390 decreased the travelled distance of mice. The two-way ANOVA of distance demonstrated a significant main effect of haloperidol ($F_{(1,24)}$=9.8809, p=0.00440) and SCH23390 ($F_{(1,24)}$=42.152, p=0.00000) (Table 1).

*p*CPA, ondansetron, WAY100635 and sulpiride did not alter locomotor activity. The two-way ANOVA of spontaneous locomotor did not demonstrate a significant difference for antagonists x FDPI interaction.

Ex vivo assays

Effect of FDPI on MAO-A and MAO-B activities

Figure 5 shows MAO-A and MAO-B activities in prefrontal cortices of mice treated with FDPI. FDPI at doses of 1, 10 or 20 mg/kg did not alter the MAO-A activity in prefrontal cortex (Figure 5A), but at doses of 10 and 20 mg/kg inhibited the MAO-B activity ($F_{(3,21)}$= 7.179, p=0.0017) (Figure 5B).

Effect of FDPI on [^{3}H]5-HT uptake

Table 2 shows the effect of FDPI on the synaptosomal [^{3}H]5-HT uptake. FDPI at all doses tested did not inhibit the synaptosomal 5-HT uptake in mice.

Discussion

The present study contributes to understanding the role of serotonergic and dopaminergic systems in the antidepressant-like action of FDPI in the FST. All doses of compound tested reduced the immobility time, the characteristic behavior measured in the modified FST. Moreover, FDPI enhanced swimming, but did not alter climbing behavior. The inhibition of MAO-B activity also appears to be involved in this effect. Importantly, the antidepressant-like activity of FDPI was not associated with any motor effects.

The FST is the most common animal model used for antidepressant drug screening studies. The modified FST over its traditional counterpart reveals that noradrenergic agents decrease immobility with a corresponding increase in climbing behavior, whereas 5-HT-related compounds such as SSRIs decrease immobility and increase swimming behavior, but do not alter the climbing behavior [19,40]. In this study, paroxetine, a well known SSRI, showed an antidepressant-like action, as expected, increasing the swimming and decreasing immobility, but without modifying the climbing behavioral. Similar to paroxetine, the results obtained in the modified FST demonstrated that the serotonergic system plays a role in the antidepressant-like effect of FDPI, excluding the possible involvement with the noradrenergic system. The fact that FDPI did not induce any significant alteration in the spontaneous locomotor activity indicates that the FDPI anti-immobility effect cannot be attributable to a psycho stimulant activity. This is a relevant result in view the fact that psychostimulant drugs may give a false positive result in animal models, such as the modified FST [41].

In order to investigate a possible contribution of the serotonergic system in the FDPI antidepressant-like action, *p*CPA was used to inhibit tryptophan hydroxylase (responsible by produced from the essential amino acid L-tryptophan in neurons) and deplete serotonin levels in mice [30,42]. The results demonstrated here indicate that the depletion of 5-HT by pCPA did not alter the activity in the FST, but antagonized the antidepressant-like effect of FDPI.

The blockade of antidepressant-like effect of FDPI by 5-HT depletion supports the hypothesis that the behavioral effects of FDPI require the serotonergic system.

To further explore the role of the serotonergic system in the FDPI antidepressant-like action, different 5-HT receptor antagonists were tested. As shown in experiments, administration of a 5-HT_{1A} receptor antagonist, WAY-100635, and a 5-HT_3 receptor antagonist, ondansetron, abolished the antidepressant-like effect of FDPI. By contrast, 5-$HT_{2A/2C}$ receptor antagonist, ritanserin, appears not be involved in the antidepressant-like effect of FDPI.

Berberine, a benzodioxoloquinolizine alkaloid, exerts an anxiolytic effect, which may be due to increased monoamines in the brain stem and to decreased serotonergic system activity, by somatodendritic 5-HT_{1A} autoreceptors and inhibition of postsynaptic 5-HT_{1A} and 5-HT_2 receptors [43]. The important role of 5-HT in depression disorder has been reported [5], and the mechanism of action of most drugs involves the inhibition of 5-HT reuptake or of monoamines degradation.

In the present study the possible action of FDPI in the 5-HT reuptake at nerve terminals in mice was carried out. An interesting finding of our study was that FDPI did not alter 5-HT reuptake in prefrontal cortex brain. Thus, the inhibited synaptosomal [^{3}H] 5-HT uptake was not responsible by the antidepressant-like effect elicited by FDPI, supporting the fact that another mechanism of action may be related to the antidepressant-like effect of the studied compound.

Furthermore, MAO, a mitochondrial protein, exists as two isoforms: MAO-A and MAO-B that differ in tissue distribution [44]. It is an enzyme important in the degradation of neurotransmitters, blocking monoamines catabolism, and consequently increasing their concentrations in the brain [45], thereby exerting antidepressant effect. The *in vitro* effect of FDPI on MAO isoforms has been demonstrated by inhibit the two isoforms [21]. Our present

result showed that a single administration of FDPI was effective to inhibit MAO-B activity, but not MAO-A activity, in prefrontal cortex of mice, which could lead to an increase in the amount of monoamines stored and released from the nerve terminals.

The involvement of dopaminergic system is also suggested in the pathophysiology of depression [46,47]. The antagonists of dopaminergic receptors were used to assess the involvement of this system in the antidepressant-like effect of FDPI.

The present study demonstrated that haloperidol, a dopamine D_2 receptor antagonist, and SCH23390, a dopamine D_1 receptor antagonist, abolished the antidepressant-like effects of FDPI. By contrast, sulpiride, a dopamine $D_{2/3}$ receptor antagonist, was not effective in blocking the antidepressant-like effect of FDPI. These results suggest that the dopaminergic system plays a role in the antidepressant-like action of FDPI. Studies demonstrate that 5-HT and DA receptors are target of isoquinolines [48,49].

In conclusion, FDPI had antidepressant-like behavior in the modified forced swim test in a pattern characteristic of serotonergic drugs. Furthermore, this study demonstrated that the FDPI effects were blocked by interaction with both serotonergic and dopaminergic systems. According to *ex vivo* results, the mechanism of action of FDPI might be related to an increase in monoaminergic activity via inhibition of monoamine oxidase-B enzyme. Nevertheless, further studies are needed to elucidate the mechanism of action and the contribution of other neurotransmission systems to antidepressant-like properties of FDPI.

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Legends of Figures

Figure 1: Chemical structure of 7-fluoro-1,3-diphenylisoquinoline -1-amine (FDPI)

Figure 2: Effects of FDPI on active behaviors in a mouse modified forced swimming test. FDPI (1 - 20 mg/kg, i.g.) was administered intragastrically to mice 30 min before the test. Paroxetine (8 mg/kg, i.p.) was intraperitoneally administered to mice 45 min before the test. Control group indicates animals administered with vehicle. Asterisk indicates groups that differ significantly from vehicle-treated animals. Each column represents mean ± S.E.M. from 6-7 animals for group. Statistical analysis was performed by one-way ANOVA followed by the Newman–Keuls test for FDPI and the Student's t-test for paroxetine (*$p < 0.05$; **$p < 0.01$; ***$p < 0.0001$ compared with the vehicle-treated control).

Figure 3. Effect of ondansetron (1 mg/kg, i.p.) (A), ritanserin (1 mg/kg, i.p.) (B), WAY100635 (0.1 mg/kg, s.c.) (C), *p*CPA (100 mg/kg i.p. once a day per 4 consecutive days) (D) and/or FDPI (1 mg/kg, p.o.) on the mouse forced swimming test. WAY100635, ritanserin or ondansetron was administered 15 min before FDPI and the forced swimming test was performed 30 min after FDPI administration. FDPI (1 mg/ kg) was intragastrically administered 24 h after the last *p*CPA administration and 30 min before the test. Each column represents mean ± S.E.M. from 6-7 animals for group. Statistical analysis was performed by two-way ANOVA followed by the Newman–Keul's test when appropriated (*) $p<0.05$ as compared with the vehicle-treated control. (#) $p<0.05$ as compared with the group pretreated with FDPI.

Figure 4. Effect of pretreatment with haloperidol (0.2 mg/kg, i.p.), SCH23390 (0.05 mg/kg, s.c.) or sulpiride (50 mg/kg, i.p.) and/or FDPI (1 mg/kg, i.g.) on the mouse forced swimming test. Antagonists were administered 15 min before FDPI and the forced swimming test was

performed 30 min after FDPI administration. Each column represents mean ± S.E.M. from 6-7 animals for group. Statistical analysis was performed by two-way ANOVA followed by the Newman–Keul's test when appropriated (*) $p<0.05$ as compared with the vehicle-treated control. (#) $p<0.05$ as compared with the group pretreated with FDPI.

Figure 5. Effect of FDPI on MAO-A and MAO-B activities in mouse prefrontal cortex. FDPI (1, 10 and 20 mg/kg) was intragastrically administered 30 min before *ex vivo* assay. Values are expressed as mean ± S.E.M of 6-7 animals/group. Statistical analysis was performed by one-way ANOVA followed by the Newman-Keul's test $*p < 0.05$ when compared to the vehicle-treated control. The values are expressed as nmol of 4- hydroxyquinoline/mg protein/min.

Figure 1

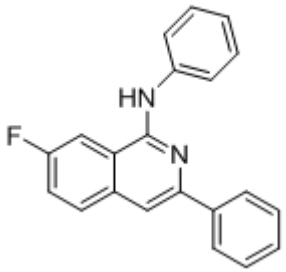
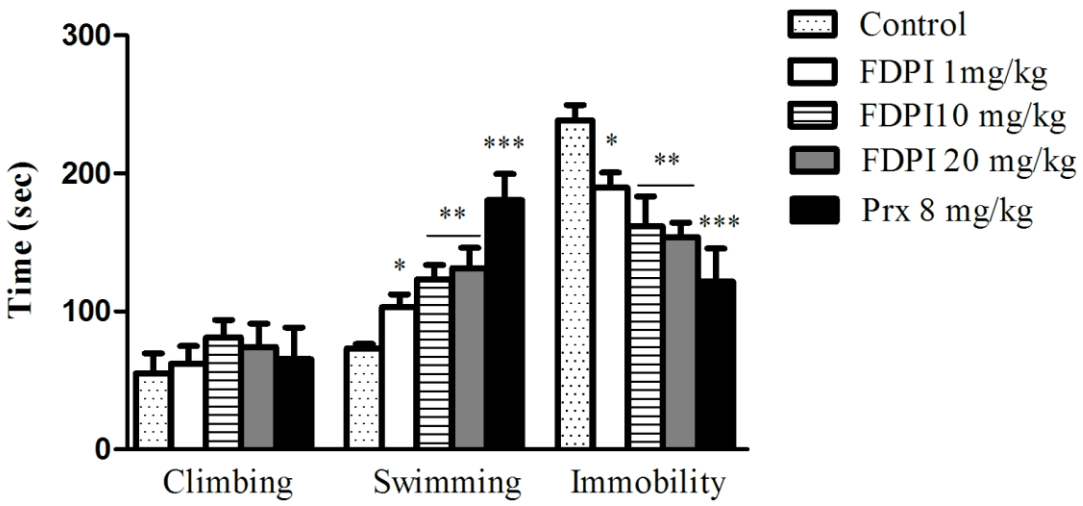


Figure 2


300
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100
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Time (sec)
Climbing
Swimming
Immobility
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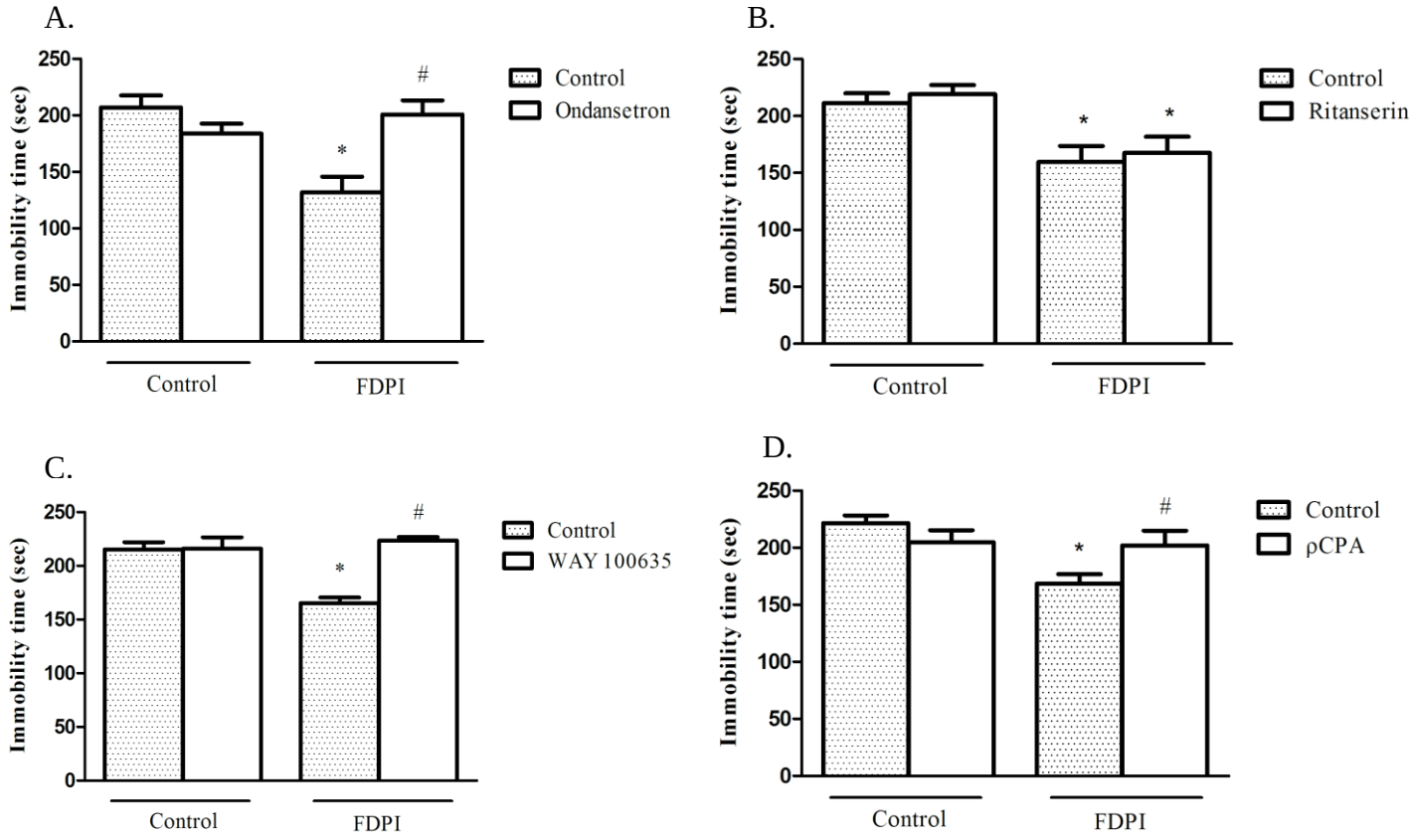
Control
FDPI 1mg/kg
FDPI10 mg/kg
FDPI 20 mg/kg
Prx 8 mg/kg

Figure 3


A.
Immobility time (sec)
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200
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FDPI
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Ritanserin
C.
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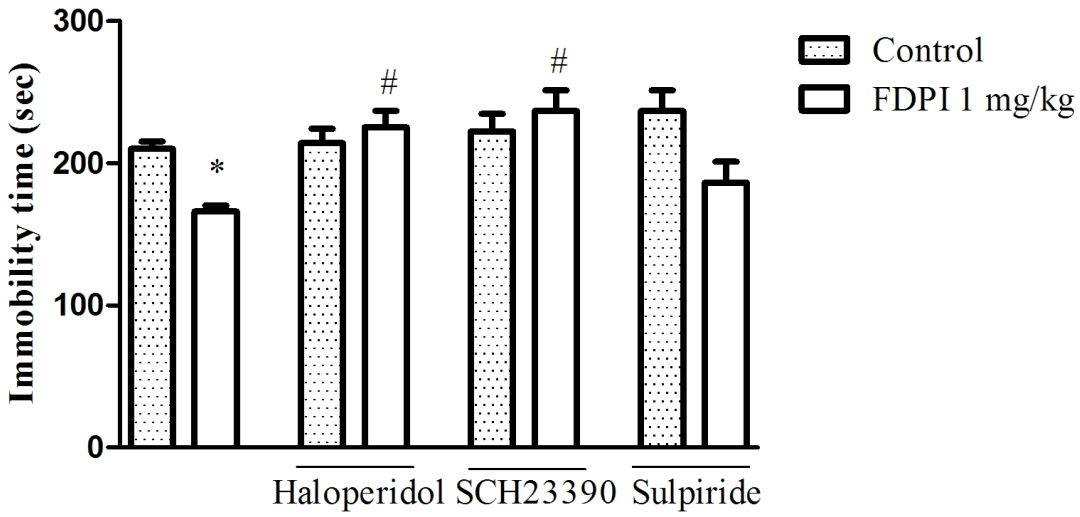
Figure 4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Control
FDPI 1 mg/kg
Haloperidol
SCH23390
Sulpiride

Figura 5

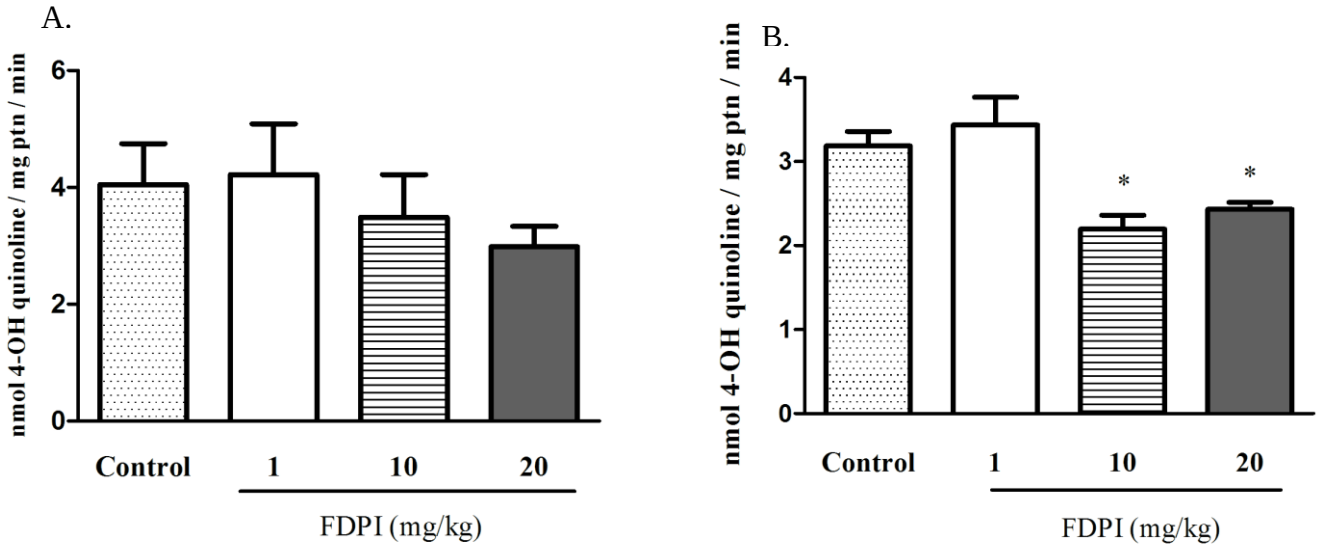
A.
nmol 4-OH quinoline / mg ptn / min
6
4
2
0
Control
1
10
20
FDPI (mg/kg)
B.
nmol 4-OH quinoline / mg ptn / min
4
3
2
1
0
*
*
Control
1
10
20
FDPI (mg/kg)

Table 1: Effect of acute administration of FDPI and its combined treatment with antagonists on the mouse spontaneous locomotor activity

Group	Crossing	Rearing	Distance	Velocity
Control	348 ± 24	10 ± 1	6032 ± 402	28 ± 2
FDPI 1 mg/kg	432 ± 23	11 ± 0.1	5683 ± 312	28 ± 2
FDPI 10 mg/kg	317 ± 61	11 ± 2	6035 ± 958	30± 2
FDPI 20 mg/kg	272 ± 40	10 ± 1	5616 ± 730	24 ± 3
Paroxetine 8 mg/kg	627 ±11*	12 ± 2	9526 ± 1458*	41 ± 6
*p*CPA	480 ± 55	16 ±1	8097 ± 873	35± 3
Ondansetron	381 ± 70	14 ± 2	7274 ± 1173	26 ± 3
Way 100635	346 ±67	13 ± 2	6362 ± 914	29 ± 3
Ritanserin	192± 43	6 ± 1	3089 ± 587	18 ± 1
Sulpiride	262 ± 9	5 ± 1	4121 ± 402	24 ± 2
Haloperidol	192 ± 22	6 ± 1	3172 ± 445	13 ± 1
SCH23390	217 ± 26	7 ± 1	3427 ± 377	18 ± 2
FDPI 1mg/kg + *p*CPA	379 ± 58	11 ± 2	6599 ± 1060	29 ± 3
FDPI 1mg/kg + Ondansetron	287 ± 40	12 ± 2	5442 ± 830	25 ± 3
FDPI 1mg/kg + Way 100635	268 ± 17	10 ± 0.9	5176 ± 334	26 ± 0.8
FDPI 1mg/kg + Ritanserin	144 ± 23	4 ± 1	2762 ± 428	24 ± 3
FDPI 1mg/kg + Sulpiride	295 ± 55	6 ± 2	5572 ± 802	26 ± 3
FDPI 1mg/kg + Haloperidol	317 ± 72	11 ± 2	4971 ± 912	23 ± 4
FDPI 1mg/kg + SCH23390	205 ± 30	7 ± 0.6	3822 ± 265	22 ± 4

FDPI (dose range 1-20 mg/kg) was administered i.g. 30 min before the test. FDPI 1 mg/kg was administered 15 min after antagonists and 30 min before the test. Paroxetine was administered i.p. 45 min before the test. Values are expressed as mean ± S.E.M of 6-7 animals/group. Statistical analysis was performed by one-way ANOVA followed by Newman‑Keuls test for FDPI, two-way ANOVA for FDPI x antagonist interaction and Student's t-test for Paroxetine. (*$p < 0.05$ compared with the vehicle-treated control).

Table 2: Effect of FDPI in the synaptosomal [3H] 5-HT uptake in mouse prefrontal cortex

Group	[^{3}H] 5-HT Uptake
Control	402.3 ± 23.49
FDPI 1 mg/kg	532.6 ± 154.4
FDPI 10 mg/kg	521.2 ± 233.4
FDPI 20 mg/kg	539.6 ± 87.65

FDPI (1, 10 and 20 mg/kg) was intragastrically administered 30 min before ex vivo assay. Values are expressed as mean ± S.E.M of 3 animals/group. Statistical analysis was performed by one-way ANOVA followed by Newman‑Keuls test. The values are expressed by *f* mol of 5-HT uptake/ mg of protein . min^{-1}

4. CONCLUSÃO

Os resultados apresentados nesta dissertação permitem concluir que o composto FDPI apresenta ação do tipo antidepressiva em camundongos no TNF modificado e esta ação é possivelmente mediada pelos sistemas serotoninérgico e dopaminérgico, além do envolvimento da inibição seletiva da monoamino oxidase-B. Desta forma, este composto derivado de isoquinolina torna-se um candidato à possível agente terapêutico para o tratamento da depressão, mediante estudos mais aprofundados em torno desta molécula.

5. PERSPECTIVAS

Tendo em vista os resultados obtidos com esse trabalho, as perspectivas para trabalhos posteriores são:

- Avaliar o efeito do FDPI em modelos de estresse crônico, bem como no estresse crônico imprevisível moderado e na separação materna;
- Realizar estudos de união específica (*binding*) entre o FDPI e os receptores serotoninérgicos.
- Avaliar o efeito do FDPI em modelos agudos de depressão (estresse de restrição);
- Investigar os possíveis mecanismos neuroquímicos de ação deste composto *in vitro*;

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