UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: BIOQUÍMICA

PARTICIPAÇÃO DOS SISTEMAS ADENOSINÉRGICO E GLUTAMATÉRGICO NO EFEITO ANSIOLÍTICO DOS DERIVADOS DA GUANINA EM RATOS.

Roberto Farina de Almeida

Orientador

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SUMÁRIO

Parte I

RESUMO

Os transtornos de ansiedade estão entre os mais prevalentes de todos os transtornos mentais no mundo. Estão associados a sofrimento subjetivo e prejuízo na qualidade de vida dos pacientes. Porém os mecanismos fisiopatológicos da ansiedade ainda necessitam de muita investigação. Recentemente, nosso grupo demonstrou que a administração sistêmica de GMP é capaz de induzir um efeito típico ansiolítico em ratos, contudo, o mecanismo de ação deste efeito ainda não foi esclarecido. Assim, no presente estudo buscamos investigar os mecanismos envolvidos no efeito ansiolítico do GMP, além de avaliar o potencial efeito ansiolítico da guanosina (GUO), e explorar o envolvimento do sistema adenosinérgico e glutamatérgico nos efeitos observados. Nossos resultados evidenciam que o GMP é capaz de promover efeito típico ansiolítico quando a administração é central, aumentar os níveis de GUO e adenosina (ADO) no liquor quando sua administração é intraperitoneal (i.p.). Ainda, observamos que a administração i.p. de GUO possui efeito típico ansiolítico, aumentando apenas os níveis de ADO no liquor. Com isso, demonstramos que a administração de GUO foi capaz de diminuir os níveis de glutamato no liquor, sem alterar a captação de glutamato em regiões do cérebro relacionadas com a ansiedade. Por fim, mostramos que o pré-tratamento com cafeína foi capaz de abolir o efeito ansiolítico da GUO e parcialmente bloquear a diminuição dos níveis de glutamato no liquor. Como conclusão, este estudo fornece novas evidências sobre o mecanismo de ação do GMP e da GUO, e mostra que o efeito ansiolítico pode estar relacionado com a modulação do sistema adenosinérgico e glutamatérgico.

ABSTRACT

Anxiety disorders represent the most common worldwide psychiatric diseases but nowadays there is no satisfactory strategy to their treatment without severe adverse effects. Recently, we demonstrated that a systemic administration of GMP induces anxiolytic-like effect in rats but its mechanism of action remains unclear. In the present study we aimed to investigate the mechanisms insights into how GMP induces anxiolytic-like effects and further explored the anxiolytic potential of guanosine (GUO) and the involvement of the adenosinergic and glutamatergic system on those effects. Our results showed that GMP induces anxiolytic-like behaviors centrally and increases cerebrospinal fluid (CSF) CSF GUO and adenosine (ADO) levels 60 min after a systemic administration. Furthermore, systemic administration of GUO produced anxiolytic-like behaviors but enhance only CSF ADO level 60 min after administration. In addition, GUO treatment decreased CSF glutamate level but did not change glutamate uptake in brain regions related to anxiety. Moreover, the pre administration of CAF abolished GUO anxiolytic-like effects and partially blocked GUO effects on CSF glutamate level. In summary, the present work presents the anxyolitic effects of GMP and GUO and provides new evidence that these effects seem to be related to the modulation of adenosinergic and glutamatergic systems.

ABREVIATURAS

- ADO, adenosina;
- ADP, adenosina difososfato;
- AMPA, α-amino-3-hidroxil-5-metil-4-isoxazole-propionato;
- AMP, adenosina monofosfato;
- AOPCP, α, ß-metilenoadenosina 5'-difosfato
- AQ, ácido quinolínico;
- ATP, adenosina trifososfato;
- AU, ácido úrico;
- CAF, cafeína;
- GDP, guanosina difosfato;
- GMP, guanosina monofosfato;
- GTP, guanosina trifosfato;
- GUO, guanosina;
- HIPOX, hipoxantina;
- i.c.v., intracerebroventricular;
- iGlu, receptor ionotrópico de glutamatp;
- INO, inosina;
- i.p., intraperitoneal;
- KA, ácido Kaínico
- mGlu, receptores metabotropicos de glutamato;
- NL, nitrogênio líquido;
- NMDA, N-metil-D-aspartato;
- PDGs, purinas derivadas da guanina;
- SNC, sistema nervoso central

- XANT, xantina.

APRESENTAÇÃO:

Esta dissertação se constitui de:

Parte I

– Introdução: fundamentação teorica que originou o trabalho.

– Objetivos: objetivos gerais que orientaram esta dissertação.

Parte II

– Materiais e Métodos e Resultados: apresentam-se sob forma e normas de artigo científico a ser submetido à revista Pharmacology Biochemistry and Behavior.

Parte III

– Discussão: comentários gerais dos resultados apresentados e a conclusão do trabalho.

– Conclusão: conclusões gerais sobre os resultados apresentados neste trabalho

– Referências Bibliográficas: refere-se as citações contidas no intem I e V desta dissertação.

INTRODUÇÃO

I.1 - Ansiedade

A ansiedade é um transtorno psiquiátrico comum na prática médica. Os transtornos de ansiedade são subdivididos em transtornos de ansiedade generalizada, transtorno do pânico, fobia social, transtorno obsessivo compulsivo e transtorno de estresse pós-traumático. Os sintomas são caracterizados por: taquicardia, sudorese e dispnéia, associados à sensação de medo, desconforto ou tensão psíquica, o que pode causar grandes sofrimentos aos pacientes (Hoffman e Mathew, 2008).

Nos seres humanos, em condições normais, a ansiedade é produzida em resposta a uma variedade de eventos e permite ao indivíduo avaliar seu ambiente, assim como realizar ajustes em seu comportamento (Simon e Gorman, 2006). No entanto, quando a ansiedade se torna excessiva é classificada como transtorno de humor.

A real prevalência epidemiológica deste transtorno é de difícil identificação, principalmente devido ao fato de que pequenas alterações nos critérios diagnósticos podem afetar os resultados. No Brasil, os transtornos de ansiedade generalizada, representam 90% dos casos associados à ansiedade em serviços de atenção primária, com prevalência de 8% sobre a população brasileira (Valença, 2005). Já nos Estados Unidos (EUA), a análise de estudos estima que os transtornos de ansiedade atinjam 15,7 milhões de pessoas.

Atualmente o crescimento das doenças de humor causam grandes preocupações para os gestores da saúde pública. Custos econômicos de pacientes com transtorno de ansiedade generalizada incluem redução da produtividade no trabalho, além de aumento nos atendimentos de emergência, nas consultas psiquiátricas, na hospitalização e na prescrição de medicamentos (Lépine, 2002).

Como determinantes para a causa da doença, os avanços nas pesquisas têm ressaltado a importância de aspectos biológicos (genéticos e fisiológicos), além de eventos estressores. Apesar dos muitos estudos relacionados à ansiedade, os mecanismos fisiopatológicos subjacentes a este transtorno permanecem relativamente obscuros. Contudo, é provável que muitos sistemas de neurotransmissores diferentes possam estar envolvidos na patofisiologia da ansiedade (Nutt e Malizia, 2001).

Acredita-se que o sistema GABAérgico, assim como o sistema serotoninérgico, sejam os principais sistemas envolvidos diretamente neste transtorno (Nutt e Malizia, 2001). Isto se deve ao fato dos benzodiazepínicos que atuam no sistema GABAérgico, assim como os inibidores seletivos da recaptação de serotonina, serem os medicamentos de escolha e amplamente usados na terapêutica da ansiedade. Porém, devido a efeitos adversos que incluem hipnótico-sedativos, propriedades músculo relaxante, prejuízo de memória, ganho de peso e síndrome de retirada têm limitado a aplicação terapêutica destes fármacos (Liberzon et al., 2003; Hamner et al., 2004; van Ameringen et al., 2004; Hoffman e Mathew, 2008). Sendo assim, a pesquisa por novos fármacos capazes de tratar a ansiedade vem sendo conduzidas. Muitas teorias e compostos têm sido testados, e dentre eles a manipulação farmacológica do sistema glutamatérgico vêm ganhando grande atenção para o tratamento desta condição. Assim, drogas que modulam o sistema glutamatérgico, são potenciais alvos terapêuticos para o desenvolvimento de fármacos ansiolíticos (Bergink et al., 2004; Chojnacka-Wojcik et al., 2001; Cryan et al., 2003; Kapus et al., 2008; Palucha e Pilc, 2007).

I.2 – Sistema glutamatérgico

O glutamato é o principal neurotransmissor excitatório do sistema nervoso central (SNC) de mamíferos sendo essencial para o encéfalo. É estimado que 50 % dos neurônios cerebrais são glutamatérgicos (Cotman et al., 1995; Ozawa et al., 1998). O glutamato está intimamente relacionado com os processos de aprendizagem e memória, além de outros eventos que envolvem plasticidade neuronal (Izquierdo et al., 2006; Ozawa et al. 1998; Segovia et al., 2001), como a resposta cerebral a estímulos externos (Schmidt et al., 2008).

A ação do glutamato é realizada através da ativação de seus receptores. Contudo, a excessiva ativação do sistema glutamatérgico, causada pelo excesso de glutamato extracelular, é altamente neurotóxica (excitotoxicidade) e está intimamente relacionada a patofisiologia tanto de doenças agudas, como doenças de crônicas do cérebro (Maragakis e Rothstein, 2006; Segovia et al., 2001), incluindo doenças neurodegenerativas, isquemia cerebral, convulsão (Lipton e Rosenberg, 1994; Maragakis e Rothstein, 2006; Meldrum, 1994; Sheldon e Robinson, 2007) e transtornos psiquiátricos (Machado-Vieira R et al., 2009).

Os receptores glutamatérgicos podem ser classificados em dois grandes grupos: ionotrópicos (iGLU), ou canais iônicos (NMDA, AMPA and KA); e metabotrópicos (mGLU), que são acoplados a sistemas de segundos mensageiros através de proteínas G (Maragakis e Rothstein, 2006; Ozawa et al. 1998; Sheldon e Robinson, 2007). Já foi observado que fármacos não competitivos, bem como antagonistas competitivos dos receptores glutamatérgicos N-metil-D-aspartato (NMDA) produzem efeitos ansiolíticos (Corbett e Dunn, 1993; Dunn et al., 1989; Plaznik, et al., 1994; Winslow et al., 1990). Porém, apesar dos efeitos sobre a ansiedade, tais compostos não são utilizados terapeuticamente devido aos seus efeitos adversos (Bergink et al., 2004;

Chojnacka-Wojcik et al., 2001; Kapus et al., 2008; Kehne et al., 1991; Plaznik et al., 1994). Além disso, já foi demonstrado que o antagonismo não competitivo dos receptores AMPA pode promover efeito ansiolítico em roedores (Kotlinska e Liljequist, 1998). Mais recentemente, vários novos compostos que atuam antagonizando os receptores metabotrópicos com alta seletividade e potência foram descobertos e seus efeitos estão sendo investigados em estudos comportamentais para avaliação de parâmetros de ansiedade (Cryan et al., 2003, Chojnacka-Wojcik et al., 2001; Palucha e Pilc, 2007; Spooren e Gasparini, 2004; Tatarczynska et al., 2001a; 2001b).

Ainda, com relação à sinapse glutamatérgica, as células gliais são capazes de modular o balanço entre os papéis fisiológico/patológico do sistema glutamatérgico, favorecendo o tônus fisiológico e atenuando a excitotoxicidade (Danbolt, 2001; Chao et al., 2010 ; Eulenburg e Gomeza, 2010; Swanson et al., 2004). A captação astrocitária de glutamato é o mecanismo mais importantes para proteger neurônios da excitotoxicidade do glutamato. Cinco subtipos de transportadores já foram caracterizados em cérebro de mamíferos: EAAT1-5 (Arriza et al., 1994; Danbolt, 2001; Chao et al., 2010 ; Eulenburg e Gomeza, 2010). A atividade destes transportadores é dependente de sódio (Tziugouris e Wadiche, 2007; Danbolt, 2001; Chao et al., 2010 ; Eulenburg e Gomeza, 2010; Duan et al., 1999), sujeita à regulação e plasticidade (Tziugouris e Wadiche, 2007; Danbolt, 2001; Chao et al., 2010 ; Eulenburg e Gomeza, 2010). Atualmente, vários estudos têm considerado que a disfunção de transportadores de glutamato pode ser o evento inicial ou parte de uma cascata implicada na patologia de doenças cerebrais (Tziugouris e Wadiche, 2007; Maragakis e Rothstein, 2004; Robinson, 2006; Sheldon e Robinson, 2007; Arriza et al., 1994, Moussa et al., 2007; Machado-Vieira R et al., 2009). Assim, drogas capazes

de modular estes transportadores podem ser promissores alvos terapêuticos para diversas doenças, inclusive os transtornos psquiátricos.

Diante de tais evidências, nosso grupo de pesquisa tem demonstrado nos últimos anos que as purinas derivadas da guanina (PDGs) são capazes de modular o sistema glutamatérgico (Frizzo et al., 2003; Lara et al., 2001; Schmidt et al., 2000, 2005, 2007).

I.3 – Purinas derivadas da guanina (PDGs)

As PDG são constituídas pelos nucleotídeos GTP, GDP, GMP e o nucleosídeo guanosina (GUO). Os nucleotídeos GTP e GDP são classicamente associados ao sistema de transmissão de sinal transmembrana via proteínas G (Gudermann et al., 1997). Porém, mais recentemente foi demonstrado que as PDGs podem atuar do lado externo da membrana plasmática celular, apresentando uma importante função fisiológica e neuromodulatória no SNC; exercendo efeitos tróficos em células neurais (Ciccarelli et al., 2001; Rathbone et al., 1999); e/ou como possível ação modulatória do sistema glutamatérgico (Baron et al.,, 1989; Burgos et al., 1998; Paz et al., 1994; Regner et al., 1998), além de exercer efeitos neuroprotetores em diferentes modelos investigados (Lara et al., 2001; Schmidt et al., 2007; Vinadé et al., 2003).

Resultados obtidos através de experimentos *in vitro* mostraram que as PDG previnem respostas celulares promovidas pelo glutamato (Baron et al., 1989; Malcon et al., 1997; Paas et al., 1996; Paz et al., 1994; Regner et al., 1998; Tasca et al., 1995; Tasca et al., 1998) e que a sua administração estimula a captação de glutamato extracelular em cultura de astrócitos e fatias de córtex cerebral de ratos jovens (Frizzo et al., 2001, 2003). Além disso, têm se demonstrado que a GUO é capaz de proteger fatias córtico-cerebrais frente a insulto de hipóxia/hipoglicemia, evento que envolve

excitotoxicidade glutamatérgica (Souza et al., 2000). Complementar a esses resultados, Cicarelli e colaboradores demonstraram que após insulto hipóxico/hipoglicêmico, culturas de astrócitos aumentam em até 140% os níveis de GUO extracelular (Ciccarelli, et al., 1999).

Já em experimentos *in vivo*, as PDG administradas de diferentes formas em roedores (intracerebroventricular (i.c.v.), intraperitonial (i.p.) e oral) foram capazes de prevenir convulsões induzidas pelo ácido quinolínico (AQ), um agonista do sistema glutamatérgico, fortalecendo a hipótese de um efeito antagônico das PDGs frente à neurotoxicidade glutamatérgica (Baron et al., 1989; Lara et al., 2001; Oliveira et al., 2004; Schmidt et al., 2000, 2005; Soares et al., 2004; Vinadé et al., 2003, 2005). Além disso, já foi demonstrado que os efeitos das PDGs frente à captação de glutamato em astrócitos, assim como a convulsão por AQ em roedores são dependentes de sua conversão a GUO, tanto sistêmica quanto central, dependendo da via de administração (Frizzo et al., 2002; Soares et al., 2004). Embora, efeitos extracelulares e comportamentais já tenham sido descritos, o exato mecanismo pelo qual as PDGs exercem seus efeitos não está completamente elucidado.

Experimentos comportamentais, realizados pelo nosso grupo, já demonstraram que a administração crônica ou aguda de GUO não afeta a locomoção (Lara et al., 2001; Vinadé et al., 2003), a função motora ou tampouco exerce efeitos sedativos nos roedores em experimentos avaliando o desempenho dos animais em tarefas como: campo aberto e rota-rod (Lara et al., 2001; Vinadé et al., 2003). Ainda, já foi demonstrado que a GUO administrada *ad libitum* na água de beber de camundongos por duas semanas foi capaz de apresentar um efeito ansiolítico no teste da placa perfurada (Vinadé et al., 2003). Mais recentemente, em um estudo que investigou especificamente a ação do GMP, nas doses de 10, 25, 50, 100 e 150mg/kg em relação

ao comportamento relacionado com à ansiedade em ratos, foi demonstrado que a administração aguda e sistêmica de GMP na dose de 50mg/kg promoveu ação ansiolítica similar à administração de diazepam 2.0mg/kg em testes clássicos de ansiedade, como o claro/escuro e o labirinto em cruz elevada, sem alterar a locomoção dos animais no teste do campo aberto (Almeida et al., 2010). Nesse mesmo estudo verificou-se que não houve diferença na concentração de purinas no liquor 60 min após a administração de GMP quando comparados com os animais que receberam salina (Almeida et al., 2010). Explicações possíveis para estes resultados no liquor devem-se ao fato de que há uma dinâmica interconversão entre os nucleotídeos e nucleosídeos do sistema purinérgico (PDGs e Purinas Derivadas da Adenina) no cérebro, e não podemos descartar a possibilidade de que os níveis de PDGs tenham uma variação antes dos 60 min de análise, e esta ser a responsável pela modulação no sistema glutamatérgico. Ainda, é importante considerar que nossos resultados apresentaram grandes variações interexperimentais ao compararmos os grupos controles. Assim, diante do dinamismo das purinas no SNC uma nova metodologia que seja mais precisa e exata deve ser implementada e o mecanismo pelo qual o GMP exerce efeito ansiolítico deve ser investigado.

OBJETIVOS

Diante dos resultados já observados, o objetivo geral desta dissertação é investigar o mecanismo de ação envolvido no efeito típico ansiolítico promovido pelo tratamento sistêmico e central com as purinas derivadas da guanina em ratos.

Objetivos específicos:

1) estabelecer um novo protocolo de coleta de liquor;
2) investigar as alterações promovidas pela administração sistêmica de GMP nos níveis de purinas no liquor;
3) investigar se o efeito típico ansiolítico da administração central de GMP depende da sua conversão a GUO;
4) verificar o efeito da administração sistêmica de GUO em parâmetros relacionados à ansiedade;
5) correlacionar o envolvimento do sistema adenosinérgico com o efeito típico ansiolítico promovido pela administração de GUO;
6) investigar a modulação no sistema glutamatérgico (concentração de glutamato no liquor, e captação de glutamato em diferentes estruturas cerebrais) após a administração de GUO.

Parte II

Artigo em preparação

Involvement of adenosinergic and glutamatergic systems in anxiolytic-like effect of guanine based purines in rats

Roberto Farina Almeida, Daniel Diniz Comasseto, Denise Barbosa Ramos, Gisele Hansel, Diogo Onofre Souza, Marcelo Ganzella.

Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil.

*Corresponding author: Roberto Farina de Almeida

Rua Ramiro Barcelos, 2600 – anexo

Zip Code: 90035-003

Porto Alegre, RS

Brazil

Phone: +55 51 33085559

Fax: +55 51 33085540

E-mails:

Roberto Farina Almeida, almeida_rf@yahoo.com.br;

Marcelo Ganzella, ganzellam@hotmail.com;

Daniel Diniz Comasseto, d.comassetto@gmail.com

Denise Barbosa Ramos, denibaramos@yahoo.com.br

Gisele Hansel, gihansel@gmail.com

Diogo Onofre Souza, diogo@ufrgs.br.

Abstract:

Anxiety disorders represent the most common worldwide psychiatric diseases but nowadays there is no satisfactory strategy to their treatment without severe adverse effects. Recently, we demonstrated that a systemic administration of GMP induces anxiolytic-like effect in rats but its mechanism of action remains unclear. In the present study we aimed to investigate the mechanisms insights into how GMP induces anxiolytic-like effects and further explored the anxiolytic potential of guanosine (GUO) and the involvement of the adenosinergic and glutamatergic system on those effects. Our results showed that GMP induces anxiolytic-like behaviors centrally and increases cerebrospinal fluid (CSF) CSF GUO and adenosine (ADO) levels 60 min after a systemic administration. Furthermore, systemic administration of GUO produced anxiolytic-like behaviors but enhance only CSF ADO level 60 min after administration. In addition, GUO treatment decreased CSF glutamate level but did not change glutamate uptake in brain regions related to anxiety. Moreover, the pre administration of CAF abolished GUO anxiolytic-like effects and partially blocked GUO effects on CSF glutamate level. In summary, the present work presents the anxyolitic effects of GMP and GUO and provides new evidence that these effects seem to be related to the modulation of adenosinergic and glutamatergic systems.

1. Introduction:

Anxiety disorders represent the most common worldwide psychiatric diseases being experienced by people of all ages. These disorders are marked by varied clinical presentations, ranging from relatively moderate symptom levels to severe functional impairment and disability (Greenberg et al., 1999; Kessler et al., 2005; Koob, 2009; Lieb, 2005; Marciniak et al., 2004; Ravindran et al., 2010; Ressler, et al., 2007).

During the past 3 decades benzodiazepines have been the first-line drugs to treat anxiety disorders (Dinan, 2006). However, they produce a number of adverse effects including sedation and cognitive impairments (Barker et al., 2004). Currently, selective serotonin reuptake inhibitors (SSRI) (Ball et al., 2005) are employed in the treatment of a wide spectrum of anxiety disorders (Kent et al., 1998). However, they also present a number of adverse effects such as restlessness, insomnia, agitation, nausea, weight gain, sexual dysfunctions, and increased suicide risk (Dinan, 2006).

Nowadays, advances in the development of new strategies for the treatment of anxiety disorders are being conducted. Recently, the role of glutamate in anxiety disorders is becoming more recognized with the belief that drugs that modulate glutamatergic function have the potential to improve the current treatment of these severe and disabling illnesses (Meldrum, 2000). Accordingly, the administration of glutamate receptors antagonists have been shown to exert an anxiolytic-like effect in rodents (Palucha et al., 2007).

Several studies support the hypothesis that guanosine-5′-monophosphate (GMP) can modulate glutamatergic neurotransmission. It has been reported that GMP is able to inhibit the binding of glutamate and its analogs to brain membrane preparations (Baron et al., 1989; Hood et al., 1990; Mendieta et al., 2005; Monahan et al., 1988; Paas et al., 1996; Sharif et al., 1981; Souza et al., 1991), to prevent cell

responses to glutamate (Aleu et al., 1999; Burgos et al., 1998; 2000 and 2003; Paz et al., 1994; Regner et al., 1998; Tasca et al., 1995; 1998 and 1999) and to stimulate glutamate uptake by astrocytes (Frizzo et al., 2003). As a result, the neuroprotective potential of GMP against glutamatergic excitotoxicity has also been reported both by in vitro as by in vivo experiments (Schmidt et al., 2007).

From these evidences, we started to investigate the anxiolytic potential of GMP. Recently, we demonstrated that a systemic administration of GMP induces anxiolytic-like effect in the light/dark and elevated plus-maze (EPM) tasks in rats (Almeida et al., 2010). These results were observed 60 minutes after a 50 mg/kg (intraperitoneal) i.p. GMP administration. Surprisingly the anxiolytic-like effect was not accompanied by an increase in GMP or its metabolites levels on cerebrospinal fluid (CSF). Of note, there was a large variance in CSF purines data among the animals.

Regarding GMP effects, it is important to highlight that some of them are dependent of its conversion to GUO. For example, the anticonvulsant, amnesic and the effect on glutamate uptake promoted by GMP are blocked when an ecto-5′-nucleotidase (enzyme that converts GMP to GUO) inhibitor is used (Frizzo et al., 2003; Saute et al., 2006; Soares et al., 2004). Additionally, some authors reported the involvement of the adenosinergic system in some of GUO effects (Ciccarelli et al., 2000).

Considering the anxiolytic potential of GMP; the aims of the present study were to investigate the mechanisms insights into how GMP induces anxiolytic-like effects and additionally evaluate the anxiolytic potential of GUO. In order to do that, we (1) established a new protocol of CSF collection to improve the measurement of purines levels with more precision and accuracy; (2) investigated the changes

promoted by systemic GMP administration on CSF purines levels; (3) investigated the effects of central GMP administration and its conversion to GUO; (4) investigated the anxiolytic potential of systemic GUO administration; (5) explored the involvement of the adenosinergic system in the anxiolytic-like effect promoted by GUO; and finally (6) evaluated the CSF glutamate concentration and brain glutamate uptake after GUO administration.

2. Materials and Methods:

2.1. Animals:

Adult male Wistar rats (60–90 days old, weighing 250–350g) were kept under a 12-hour light/dark cycle (light on at 7:00 AM) at constant temperature of 22 ± 1°C. They were housed in plastic cages (5 per cage) with water and commercial food *ad libidum*. All behavioral procedures were conducted between 10:00 AM and 5:00 PM. All procedures were carried out according to the institutional policies on animal experimental handling designed to minimize suffering of animals used and the procedures were according to the Brazilian Society for Neuroscience and Behavior's recommendations for animal care.

2.2. Chemicals:

GMP, GUO, α, ß-methyleneadenosine 5'-diphosphate (AOPCP), and caffeine (CAF) were obtained from Sigma Chemicals (St. Louis, MO, USA). L-[^{3}H] glutamate (specific activity 30 Ci/mmol) was from Amersham International, UK. The anesthetic sodium thiopental was obtained from Cristália (Itapira, SP, Brazil). GMP, GUO and CAF were dissolved in saline (NaCl 0.9%).

2.3. Treatments

Animals were handled carefully in the testing room during the 6 days preceding the experiment. At the day of behavioral task, the animals were acclimatized in the testing room for 1 hour before the treatments.

Each animal was used once, as it was already demonstrated that 2 sequential experiments with the same animal promoted behavioral disturbances in anxiety-related behaviors (Almeida et al., 2010; Carobrez and Bertoglio, 2005).

In i.p. treatments rats received 1 ml/kg injection.

2.3.1. Treatment I – In the first set of experiments the animals were treated with an i.p. injection of saline and 60 min after the CSF were collected for purines evaluation. After collected the CSF samples were frozen by two different methods that are described below (section 2.6.1.1)

2.3.2. Treatment II – In the second set of experiments we administered GMP 50mg/kg in order to determine whether GMP i.p. administration is able to modulate CSF purines levels 60 min after injection. The time of administration and dose of GMP were chosen based upon previous data (Almeida et al., 2010).

2.3.3. Treatment III – In order to investigate if GMP could promote anxiolytic-like behaviors centrally and if GMP conversion to GUO was important to the related effect, we evaluate the intracerebroventricular (i.c.v.) GMP administration and the influence of the pretreatment with AOPCP, an ecto-5'-nucleotidase inhibitor, on anxiety-related parameters. Firstly we implanted a guide cannula through a stereotaxic surgery. After 48 hours of recovery, we administered an i.c.v. pretreatment with 5µl of saline or AOPCP (12.5nmol). After three minutes we treat the rats with 4 µl i.c.v. injection of GMP (960nmol) or saline and exposed the animals 5 minutes later to EPM task. The AOPCP and GMP doses were dose chosen based upon previous data analyzing a dose curve for each of the tasks performed in this study - data not shown.

2.3.4. Treatment IV – In search to analyze whether a direct administration of GUO has an anxiolytic effect, in the fourth set of experiments the animals were divided in different groups: saline, GMP 50mg/kg and GUO (7.5, 30 and 60mg/kg). Sixty minutes after the i.p. treatments the animals were submitted to the EPM task then the CSF were immediately collected.

2.3.5. Treatment V – In the fifth set of experiments, to investigate the involvement of the adenosinergic and glutamatergic system in the anxiolytic-like effect exerted by GUO, we divided the rats on four groups (Sal/Sal; CAF10mg/kg/Sal; Sal/GUO7.5mg/kg; CAF10mg/kg/GUO7.5mg/kg). The saline or CAF pretreatment were administered i.p. 15min before the saline or GUO treatment. Sixty min after the treatments animals were submitted to EPM task then anesthetized and the CSF collected. After, the animals were decapitated, their brains immediately removed and the hippocampus, amygdala and prefrontal cortex were dissected onto Petri dishes for further analysis of glutamate uptake. The CAF dose was chosen based upon previous data analyzing a dose that did not change any behavioral of rats (data not shown).

2.4. Elevated Pluz Maze (EPM) tasks

The EPM was performed as previously described (Pellow et al., 1986). The EPM apparatus, entirely made of wood, consisted of two open arms (50 x 10 cm, length x width) and two enclosed arms (50 x 10 x 40 cm; length x width x height) separated by a central platform (5 x 5 cm; length x width) arranged so that the two identical arms of each type were opposite to each other. The height of the maze was 70 cm, and the experiments were conducted under dim red light (25lux) in a quiet room. The animals were placed individually on the central platform of the maze facing an open arm, and recorded individually for 5 minutes by AnyMaze Software®. After the behavioral section the videos were observed by a blind to treatment researcher. The %

time in open arms, the % entries in the open arms and the total transitions into the apparatus were analyzed. Only when there was an statistical augmentation in the first two items cited above the treatment was considered anxiolytic. Locomotor impairment is considered when there is a decrease in the total transitions into the apparatus. After each session the apparatus was cleaned with alcohol 70° and dried before the next animal.

2.5. Surgical Procedure

Surgery and i.c.v. infusion techniques were adapted from Schmidt et al., 2000 (Schmidt et al., 2000). Animals were anesthetized with sodium thiopental (60 mg/kg, 1 ml/kg, i.p.). In a stereotaxic apparatus, the skin of the skull was removed, and an i.c.v. guide cannula for infusion was implanted. Stereotaxic coordinates were 0.9 mm posterior to bregma, 1.5 mm right of the midline. The guide cannula was implanted 2.6 mm ventral to the superior surface of the skull and fixed with jeweler's acrylic cement. Experiments were performed 48 h after surgery. I.c.v. treatments were performed with a 30-gauge cannula which was fitted into the guide cannula and connected by a polyethylene tube to a micro syringe. The tip of the infusion cannula protruded 1 mm beyond the guide cannula aiming the right lateral brain ventricle.

2.6. Cerebrospinal Fluid (CSF) analysis

2.6.1. CSF Sampling.

For CSF collection, the rats were anesthetized with sodium thiopental (40mg/kg, 1 ml/kg, i.p.), and placed in a stereotaxic apparatus. The CSF was collected (100 to 150 µl) by direct puncture of the cisterna magna with an insulin syringe (27 gauge x 1/2-inch length). Individual samples with visible blood contamination were

discarded. All samples were centrifuged at 10.000 g at 4°C in an Eppendorf centrifuge for 10 min to obtain cell-free supernatants.

2.6.1.1 CSF freezing

After collected the CSF samples were frozen by two different methods:

Method I: the samples were maintained on ice until the end of the experiment (the first samples were maintained on ice for approximately 3 hours while the last was kept for only few minutes) when they were stored in -80°C freezer, as performed in the (Almeida et al., 2010).

Method II: the CSF samples were immediately frozen after collected with liquid nitrogen (LN) and stored in -80°C freezer.

We applied only the LN fast freezing method when CSF was collected in the other treatments regimen.

2.6.2. High-performance liquid chromatography (HPLC) for purines analysis

HPLC was performed with aliquots obtained from the CSF cell-free supernatants. The measurement was done as described previously (Schmidt et al., 2009). The levels of the following purines were determined: adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), adenosine (ADO), guanosine triphosphate (GTP), guanosine diphosphate (GDP), guanosine monophosphate (GMP), guanosine (GUO), inosine monophosphate (IMP), inosine (INO) , hypoxanthine (HIPOX), xanthine (XANT) and uric acid (UA). Analyses were performed with the Shimadzu Class-VP chromatography system, consisting of a quaternary gradient pump with vacuum degassing and piston desalting modules, Shimadzu SIL-10AF auto injector valve with 50 mL loop and a UV detector (Shimadzu, Kyoto, Japan). Separations were achieved on a Supelco 250 mm x 4.6

mm, 5 µm particle size column (Supelco, St Louis, MO, USA). The mobile phase flowed at a rate of 1.2 mL/min and the column temperature was 24°C. Buffer composition remained unchanged (A: 150 mmol/L phosphate buffer, pH 6.0, containing 150 mmol/L potassium chloride; B: 15% acetonitrile in buffer A). The gradient profile was modified to the following content of buffer B in the mobile phase: 0% at 0.00 minutes, 2% at 0.05 minutes, 7% at 2.45 minutes, 50% at 10.00 minutes, 100% at 11.00 minutes, and 0% at 12.40 minutes. Samples of 25 µl were injected into the injection valve loop. Absorbance was read at 254 nm. CSF concentrations of purines are expressed as µMolar (as mean±SEM).

2.6.3. HPLC for glutamate analysis

HPLC for glutamate analysis was performed with CSF cell-free supernatant aliquots according to Schmidt et al., 2009 (Schmidt et al., 2009). Briefly, samples were derivatized with o-phthalaldehyde and separation was carried out with a reverse phase column (Supelcosil LC-18, 250 mm× 4.6 mm, Supelco) in a Shimadzu Instruments liquid chromatograph (50 µL loop valve injection). The mobile phase flowed at a rate of 1.4 mL/min and column temperature was 24°C. Buffer composition is A: 0.04 mol/L sodium dihydrogen phosphate monohydrate buffer, pH 5.5, containing 20% of methanol; B: 0.01 mol/L sodium dihydrogen phosphate monohydrate buffer, pH 5.5, containing 80% of methanol. The gradient profile was modified according to the content of buffer B in the mobile phase: 0% at 0.00 min, 25% at 13.75 min, 100% at 15.00– 20.00 min, 0% at 20.01–25.00 min. Absorbance was read at 360 nm and 455 nm, excitation and emission respectively, in a Shimadzu fluorescence detector. Samples of 50 µL were used and concentration was expressed in µMolar (as mean±SEM).

2.7. Glutamate uptake

Glutamate uptake was assessed as fully detailed elsewhere (Frizzo et al., 2002). From each animal, 6 slices of different brain structures (hippocampus, amygdala and prefrontal cortex) were used. The slices were separated in 2 equal sets, placed into separated 24-well culture plates; one plate was maintained at 37°C and the other on ice, for evaluation of total and Na^+-independent glutamate uptake, respectively, with Hank's Buffered Salt Solution (HBSS) containing (in mM): 137 NaCl; 0.63 Na_2HPO_4; 4.17 $NaHCO_3$; 5.36 KCl; 0.44 KH_2PO_4; 1.26 $CaCl_2$; 0.41 $MgSO_4$; 0.49 $MgCl_2$ and 5.5 glucose (pH 7.2). For the total glutamate uptake, the slices were washed once with 1 mL of 37°C HBSS and then pre-incubated at 37°C for 15 min, followed by the addition of 0.33 Ci/mL L-[^{3}H]glutamate and 100 uM (final concentration) glutamate. The incubation time was structure specific. The incubation was stopped after 5 min for hippocampus and amygdala, and 7 min for prefrontal cortex with 2 ice-cold washes of 1mL HBSS, immediately followed by the addition of 0.5N NaOH, which were then kept overnight. Na^+-independent uptake was measured using the same protocol described above, with differences in the temperature (4°C) and medium composition (N-methyl-d-glucamine instead of sodium chloride). Results (Na^+-dependent uptake) were considered as the difference between both uptakes. The incorporated radioactivity was measured in a Hidex 300 SL scintillation counter. After radioactivity analysis, the samples were normalized by the amount of proteins.

2.8. Protein determination

Protein concentration was determined by the method of Lowry (Lowry et al., 1951). Bovine serum albumin was used as standard.

2.9. Statistical analysis

Unpaired Student's t test was used for analyzing the experiments where we compared only two different groups. One way ANOVA followed by Tukey post hoc test was used to analyze the anxiolytic-like effects of different doses of GUO. Two way analysis of variance (ANOVA) followed by Bonferroni post-hoc test was used to compared the influence of the AOPCP pre-treatment on GMP (factors were pre treatment with APOCP and treatment with GMP) and also the influence of CAF pre-treatment on GUO effects (factors were pre treatment with CAF and treatment with GUO).

3. Results:

3.1. Fast freezing with liquid nitrogen preserves CSF nucleoside levels

In table 1 we observed that CSF samples immediately frozen in LN had a significant increase in CSF ADO, INO and HIPOX levels, and a significant decrease in XANT level compare to CSF samples only frozen in the end of experiment in -80°C freezer.

The CSF levels of the nucleotides AMP; IMP; GDP did not show any statistical differences (data not show). Importantly, CSF levels of ATP, ADP, GTP and GMP were not detectable in any CSF sample analyzed in this work.

3.2. GMP increases CSF GUO and ADO levels

In Fig. 1 Sixty min after GMP injection there was a statistical significantly increase of CSF GUO (A) and ADO (B) levels ($P \leq 0.01$; 0.05 respectively) compared to saline treated animals. Additionally, no other difference in the others CSF purines levels were observed ($P > 0.05$, data no shown).

3.3. Central GMP administration induces anxiolytic-like behaviors

The results showed in Fig. 2 demonstrated that i.c.v. injected Sal/GMP 960 nmol increased (A) % time in open arms, (B) % open arms entries ($P \leq 0.001$) without any statistical difference on locomotor activity (C). No statistical differences were observed when rats were treatment with AOPCP/sal.

On the other hand, AOPCP/GMP treatment increased significantly the (A) % time in open arms ($P \leq 0.05$), without any statistical difference in the % open arms entries. However, there was a significant locomotor impairment (C) ($P < 0.05$).

3.4. Systemic GUO administration induces anxiolytic-like behaviors

The results presented in Fig. 3 (A) shows that animals treated with GMP 50mg/kg or GUO 7.5mg/kg presented a significant enhancement in the % time in open arms ($P<0.01$). Moreover, (B) GMP 50mg/kg, GUO 7.5 or 60mg/kg statistically increased the % of entries into the open arms of the apparatus ($P<0.05$). Additionally, we did not observe any differences in the total transition on the apparatus (Fig. 1C).

3.5. GUO administration increases CSF ADO but not GUO levels

There was a statistical increase but no change in the CSF (Fig. 4B) ADO ($P<0.01$) and (Fig. 4A) GUO level, respectively, 60 min after i.p. administration of GUO 7.5mg/kg.

3.6. CAF abolish the anxiolytic-like effect promoted by GUO

The animals that received i.p. injection of Sal/GUO 7.5mg/kg presented a significantly enhancement in % time in open arms (Fig. 5A, $P<0.01$) and in % of entries into the open arms (Fig. 5B, $P<0.01$). However, the pretreatment with CAF statistically abolished the GUO anxiolytic-like effects.

Additionally, no differences were observed with the CAF/Sal group in any parameter analyzed. Moreover, none of the experimental groups change the total transitions in the EPM apparatus (Fig. 5C, $P > 0.05$).

3.7. CAF partially blocks the decrease of CSF glutamate level promoted by GUO

In fig. 6 we observed that Sal/GUO significantly decrease CSF glutamate level (Fig.6 $P<0.05$). Additionally, the pre-administration of CAF partially blocked the decrease of CSF glutamate level promoted by GUO.

3.8. Glutamate Uptake

There were no statistical differences on glutamate uptake in any brain structure investigated (hippocampus, amygdala and pre-frontal cortex) among the experimental CAF, GUO or CAF/GUO groups 60 min after treatment (data not show).

4. Discussion:

In the present article we extended the investigation about the anxiolytic potential of the guanine based purines (GBPs) and additionally provided some evidences of their mechanisms of action.

Previously, we already had shown that a systemic GMP administration induces anxiolytic-like effects in rats but these effects were not accompanied by increasing CSF purines levels (Almeida et al., 2010). Firstly, we argued that one possibility would be that changes in CSF purines levels could occur before the time investigated (60 minutes after administration) which would explain the anxiolytic-like effect. This argument was emphasized by one previous work which showed that a systemic GMP administration was able to increase CSF GUO but not GMP or ADO levels 30 min after injection (Soares et al., 2004). However, the data regarding the CSF purines content from our previous work claimed our attention about the high variability among the samples.

Of note the purinergic system presents a high rate of metabolization in the brain, including CSF (Burnstock, G. 2002; Cunha, 2001; Silva et al., 2004; Zimmermann, 2006a). It was already described the presence of several enzymes responsible to metabolize and also to interconvert the nucleotides/nucleosides of the purinergic system (Schmidt et al., 2007). These points lead us to speculate that the high variability of our data could be influenced by the action of these enzymes after the CSF collection.

In this order we applied a new methodology based on freezing immediately the samples with LN in contrast to the used in our previous work, where we did not freeze before stored at -80ºC. As show in table 1, the fast freezing with LN preserves the nucleoside content (ADO/INO/HIPOX) thus decreasing their metabolites (XAN/UA). Accordingly freezing the CSF immediately after collection is better suitable for purines levels measurement. This data is reinforced when we compared our present results with others previous studies which did not fast frozen the CSF samples after collection. For example, the CSF GUO levels varied among the previous studies from approximately 0.1 to 1 μM (Soares et al., 2004; Schmidt et al., 2010; Vinadé et al., 2005).

Thus, after implementing a methodology that reduced the variation in CSF purines levels, our first strategy was to re-evaluate the CSF purines levels 60 min after an i.p. GMP administration. At this time we could observe that both CSF GUO and ADO levels were increased after systemic GMP administration (Fig. 1). In addition, GMP levels continuous not detectable. This result suggested to us that the anxiolytic-like effect was not directly mediated by GMP but instead GMP has to be converted to GUO.

Taken these new data, we further intended to investigate if the anxiolytic-like effects promoted by GMP could be central and involves a centrally conversion of GMP to GUO as it was already described for others GMP effects as amnesic and anticonvulsant (Saute et al., 2006; Soares et al., 2004). As shown in Fig. 2, an i.c.v GMP administration induces anxiolytic-like behavior. Unexpectedly, when we administered i.c.v. AOPCP plus GMP there was a significantly impairment on locomotor activity of the animals. As the parameters used to measure anxiety-related behavior are strictly related to the locomotor ability of the animals (Carobrez and Bertoglio, 2005), it was impossible to use AOPCP to investigate the dependence of the central GMP conversion to GUO on anxiolytic-like effects. Although we did not explore in the present study the interaction between AOPCP and GMP this need to be investigate in future studies. Additionally, the impairment on locomotor activity observed after AOPCP/GMP administration seems to not compromise the protocols used to investigated the others GMP effects as amnesic and anticonvulsant.

Given these results, we investigated the effects of a direct i.p. administration of GUO. As shown in Fig. 3, a systemic GUO 7.5 but not 30 or 60mg/kg administration was able to induce anxiolytic-like behaviors in rats on EPM task similar to GMP. Interestingly, both GMP and GUO administration were able to increase CSF ADO level, but surprisingly only GMP increased CSF GUO level (Fig. 4). These data led us to hypothesize that ADO could be involved with GBPs anxiolytic-like effects.

Although there are several GBPs effects that do not involve the adenosinergic system (Lara et al., 2001; Roesler et al., 2000; Vinade et al., 2004) there are few already described that is related to ADO. Accordingly, Ciccarelli and colleagues observed that GUO stimulates the release of ADO from astrocytes, which may in turn participate on trophic effects promoted by GUO (Ciccarelli et al., 2000). Moreover,

our group demonstrated that the antinociceptive GUO effects in mice seem to be related to the modulation of inhibitory A1 and facilitatory A2A receptors (Schmidt et al., 2010).

Furthermore, the adenosinergic system seems to be closely related with the modulation of anxiety disorders. It is known that ADO mediate inhibition of synaptic transmission by decreasing stimulus-evoked release of glutamate in central synapses by activation of A1 receptors (Ambrósio, et al., 1997; Wu and Saggau, 1994; Yawo and Chuhma, 1993). As decreasing glutamate neurotransmission seems to be an effective anxiolytic strategy (Simon and Gorman, 2006; Steckler et al., 2005; Swanson et al., 2005), it has been already demonstrated that A1 receptors agonists are effective for anxiety (Florio et al., 1998, Jain et al., 1995). Moreover, Johansson and collaborators showed that A1 receptors KO mice presents increases in anxiety-related behaviors (Johansson et al., 2001).

Altogether, in order to investigate the involvement of ADO on the anxiolytic-like effect of GUO, we pretreated the animals with CAF (an unspecific ADO receptors antagonist) and evaluated the anxiety related behavior 60 min after GUO administration. CAF completely abolished the GUO anxiolytic-related effect (Fig. 5) thus indicating that the anxiolytic potential of GUO involves the adenosinergic system.

Apart from ADO, GUO by itself can also modulate glutamatergic neurotransmission. Our group already evidenced that GUO enhance astrocytic glutamate uptake when extracellular glutamate level is elevated (Frizzo et al., 2002). Therefore we evaluated the CSF glutamate level and glutamate uptake in slices of brain regions related to anxiety.

Our results showed that GUO significantly decreased CSF glutamate level but it did not change glutamate uptake. On the one hand, these data reinforce the previous

hypothesis from our group that modulation of glutamate uptake by GUO only occurs in excitotoxicity conditions (Frizzo et al., 2002). On the other hand, we can speculate with these data that GUO could modulate the glutamatergic tonus indirectly by increasing CSF ADO level thus decreasing glutamate release.

Concerning this hypothesis, we explored where a CAF pretreatment could inhibit GUO effects on CSF glutamate level. As showed in Fig. 6, CAF partially blocked the decrease on CSF glutamate level promoted by GUO. Therefore the anxiolytic potential GUO seems to involve ADO effects on glutamate release.

Summarizing, the present work clearly demonstrate the anxiolytic potential of GBPs and further provide evidences of the involvement of the adenosinergic and glutamatergic system on these effects. Because GBPs is an endogenous compound apparently well tolerated and orally active, it could eventually be developed as a drug useful for managing anxiety. Additionally, we are continuing to investigate the mechanisms involved in the anxiolytic potential of GBPs and the mechanism of action.

Legends:

Table 1 – Fast freezing with liquid nitrogen preserves CSF nucleoside levels. CSF was collected 60 min after i.p. saline administration and frozen by two different methods for purines analyses, as described in section 2.6.1.1 (Method I e Method II). Data are expressed as mean ± SD and were analyzed by Unpaired Student's t test (n = 5 - 8 per group).* $P<0.05$ compared to Method I group.

Figure 1 - GMP increases CSF GUO and ADO levels.

CSF GUO (A) and ADO (B) levels were measured 60 min after an i.p. injection of saline or GMP 50mg/kg. Data are reported by mean ± S.E.M. and were analyzed by

Unpaired Student's t test (n = 7 animals per group). * P<0.05 compared to saline group.

Figure 2 - Central GMP administration induces anxiolytic-like behaviors.

The % time in open arms (A), % open arms entries (B) and total transitions on the apparatus (C) were evaluated 5 min after treatments on EPM task. Animals received an i.c.v. pretreatment with saline or AOPCP (12,5 nmol) 3 min before i.c.v. infusion of saline or GMP (960nmol). Data are expressed by mean ± S.E.M. Differences among groups were determined by two-way analysis of variance (ANOVA) followed by Bonferroni post test when applicable (n = 8-10 animals for each group). *P<0.05 and ***P<0.001 compared to saline/saline group.

Figure 3 - Systemic GUO administration induces anxiolytic-like behaviors

The % time in open arms (A), % open arms entries (B) and total transitions of the apparatus were evaluated 60 min after i.p. GMP 50 mg/kg or GUO 7.5, 30, 60 mg/kg administration on EPM task (C). Data are reported as mean ± S.E.M and were analyzed by one-way ANOVA, followed by Tukey's Multiple Comparison post test (n=10 – 14 rats per group), * P<0.05 and **P<0.01 compared to saline group.

Figure 4 - GUO administration increases CSF ADO but not GUO levels

CSF GUO (A) and ADO (B) levels were measured 60 min after an i.p. injection of saline or GUO (7.5mg/kg). Data are reported by mean ± S.E.M. and were analyzed by Unpaired Student's t test (n = 10 – 14 animals per group). **P<0.01 compared to saline group.

Figure 5 – CAF abolish the anxiolytic-like effect promoted by GUO

The % time in open arms (A), % open arms entries (B) and total transitions on the apparatus (C) were evaluated 60 min after treatments on EPM task. Animals received an i.p. pretreatment with saline or CAF (10 mg/kg) 15 min before i.p. injection of

saline or GUO (7.5 mg/kg). Data are reported as mean ± S.E.M and differences among groups were determined by two-way analysis of variance (ANOVA) followed by Bonferroni post test when applicable (n = 12-16 animals for each group). **P<0.01 compared to saline/saline group.

Figure 6 - CAF partially blocks the decrease of CSF glutamate level promoted by GUO

CSF glutamate level was measured 60 min after the treatments. Animals received an i.p. pretreatment with saline or CAF (10 mg/kg) 15 min before i.p. injection of saline or GUO (7.5 mg/kg). Data are reported as mean ± S.E.M and differences among groups were determined by two-way analysis of variance (ANOVA) followed by Bonferroni post test when applicable (n = 12-16 animals for each group). *P<0.05 compared to saline/saline group.

5. References:

1. Aleu J, Barat A, Burgos JS, Solsona C, Marsal J, Ramirez G. Guanine nucleotides, including GMP, antagonize kainite responses in Xenopus oocytes injected with chick cerebellar membranes. J Neurochem 1999; 72:2170–2176.
2. Almeida RF, Cereser VH, Faraco RB, Böhmer AE, Souza DO, Ganzella M. Systemic administration of GMP induces anxiolytic-like behavior in rats. Pharmacol Biochem and Behav 2010; 96:306-11.
3. Ambrósio AF, Malva JO, Carvalho AP, Carvalho CM. Inhibition of N-, P/Q- and other types of Ca2+ channels in rat hippocampal nerve terminals by the adenosine A1 receptor, Eur. J. Pharmacol 1997; 340:301–310.
4. Ball SG, Kuhn A, Wall D, Shekhar A, Goddard AW. Selective serotonin reuptake inhibitor treatment for generalized anxiety disorder: a double-blind,

prospective comparison between paroxetine and sertraline. J Clin Psychiatry 2005; 66:94−99.

5. Barker MJ, Greenwood KM, Jackson M, Crowe SF. Cognitive effects of long-term benzodiazepine use: a meta-analysis. CNS Drugs 2004; 18:37−48.
6. Baron BM, Dudley MW, McCarty DR, Miller FP, Reynolds IJ, Schmidt CJ. Guanine nucleotides are competitive inhibitors of N-methyl-D-aspartate at its receptor site both in vitro and in vivo. J Pharmacol Exp Ther 1989; 250:162–169.
7. Burgos JS, Barat A, Souza DO, Ramirez G. Guanine nucleotides protect against kainate toxicity in an ex vivo chick retinal preparation. FEBS Lett 1998; 430:176–180.
8. Burgos JS, Barat A, Ramirez G. Guanine nucleotides block agonist-driven 45Ca2+ influx in chick embryo retinal explants. Neuroreport 2000; 11:2303–2305.
9. Burgos JS, Aleu J, Barat A, Solsona C, Marsal J, Ramirez G. Kainate-triggered currents in Xenopus oocytes injected with chick retinal membrane fragments: effect of guanine nucleotides. Invest Ophthalmol Vis Sci 2003; 44:3124–3129.
10. Burnstock, G. Purinergic signaling: an overview. Novartis Found Symp 2006b; 276:26−48.
11. Carobrez AP, Bertoglio LJ. Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. Neurosci Biobehav Rev 2005; 29:1193-205.
12. Ciccarelli R, Di Iorio P, D'Alimonte I, Giuliani P, Florio T, Caciagli F, Middlemiss PJ, Rathbone MP. Cultured astrocyte proliferation induced by

extracellular guanosine involves endogenous adenosine and is raised by the co-presence of microglia. Glia 2000; 29:202-11.

13. Cunha, RA.Regulation of the ecto-nucleotidase pathway in rat hippocampal nerve terminals. Neurochem Res 2001; 26:979–991.
14. Dinan, T. Therapeutic options: addressing the current dilemma. Eur Neuropsychopharmacol 2006; 16:119−127.
15. Florio C, Prezioso A, Papaioannou A, Vertua R. Adenosine A1 receptors modulate anxiety in CD1 mice. Psychopharmacology 1998; 136:311-319.
16. Frizzo ME, Lara DR, Prokopiuk Ade S, Vargas CR, Salbego CG, Wajner M, Souza DO. Guanosine enhances glutamate uptake in brain cortical slices at normal and excitotóxico conditions. Cell Mol Neurobiol 2002; 22:353–363
17. Frizzo ME, Antunes Soares FA, Dall'Onder LP, Lara DR, Swanson RA, Souza DO. Extracellular conversion of guanine-based purines to guanosine specifically enhances astrocyte glutamate uptake. Brain Res 2003; 972:84–89.
18. Greenberg PE, Sisitsky T, Kessler RC, Finkelstein SN, Berndt ER, Davidson JR, Ballenger JC, Fyer AJ. The economic burden of anxiety disorders in the 1990s. J Clin Psychiatry. 1999; 60:427-435.
19. Hood WF, Thomas JW, Compton RP, Monahan JB. Guanine nucleotide modulation of [3H]TCP binding to the NMDA receptor complex. Eur J Pharmacol 1990; 188:43–49
20. Jain N, Kemp N, Adeyemo O, Buchanan P, Stone TW Anxiolytic activity of adenosine receptor activation in mice. Br J Pharmacol 1995; 116:2127-2133.
21. Johansson B, Halldner L, Dunwiddie TV, Masino SA, Poelchen W, Giménez Llort L, Escorihuela RM, FernándezTeruel A, Wiesenfeld-Hallin Z, Xu XJ, Hrdemark A, Betsholtz C, Herlenius E, Fredholm BB. Hyperalgesia, anxiety,

and decreased hypoxic neuroprotection in mice lackingthe adenosine A1 receptor. Proc Natl Acad Sci 2001; 98:9407-9412.

22. Kent, J.M., Coplan, J. D., & Gorman, J.M.. Clinical utility of the selective serotonin reuptake inhibitors in the spectrum of anxiety. Biol Psychiatry 1998; 44, 812−824.
23. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry 2005; 62:593–602
24. Koob, G. F. Brain stress systems in the amygdala and addiction. Brain Res. 2009; 1293:61–75
25. Lara, D. R., Schmidt, A. P., Frizzo, M. E. S., Burgos, J. S., Ramirez, G., & Souza, D. O. Effect of orally administered guanosine on seizures and death induced by glutamatergic agents. Brain Res 2001; 912:176−180
26. Lieb, R. Anxiety disorders: clinical presentation and epidemiology. Handb. Exp. Pharmacol. 2005; 169:405–432
27. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:265–275
28. Marciniak M, Lage MJ, Landbloom RP, Dunayevich E, Bowman L. Medical and productivity costs of anxiety disorders: case control study. Depress Anxiety 2004; 19:112-120.
29. Meldrum BS. Glutamate as a neurotransmitter in the brain: review of physiology and pathology. J Nutr 2000; 130:1007S-1015S

30. Mendieta J, Gago F, Ramirez G.Binding of 5′-GMP to the GluR2 AMPA receptor: insight from targeted molecular dynamics simulations. Biochemistry 2005; 44:14470–14476.
31. Monahan JB, Hood WF, Michel J, Compton RP. Effects of guanine nucleotides on N-methyl-D-aspartate receptor-ligand interactions. Mol Pharmacol 1988; 34:111–116.
32. Paas Y, Devillers-Thiery A, Changeux JP, Medevielle F, Teichberg VI. Identification of an extracellular motif involved in the binding of guanine nucleotides by a glutamate receptor. EMBO J 1996; 15:1548–1556.
33. Paz MM, Ramos M, Ramirez G, Souza DO. Differential effects of guanine nucleotides on kainic acid binding and on adenylate cyclase activity in chick optic tectum. FEBS Lett 1994; 355:205–208.
34. Palucha, A, Pilc, A. Metabotropic glutamate receptor ligands as possible anxiolytic and antidepressant drugs. Pharmacology & Therapeutics 2007; 115; 116 – 147.
35. Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J of Neurosci Methods 1986; 14:149–67.
36. Ravindran LN, Stein MB. Pharmacotherapy of post-traumatic stress disorder. Curr Top Behav Neurosci 2010; 2:505-25.
37. Regner A, Ramirez G, Bello-Klein A, Souza D. Effects of guanine nucleotides on glutamate-induced chemiluminescence in rat hippocampal slices submitted to hypoxia. Neurochem Res 1998; 23:519–524.

38. Ressler, KJ; Mayberg, HS. Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. Nature Neurosci 2007; 10, 1116–1124

39. Roesler R, Vianna MR, Lara DR, Izquierdo I. Schmidt AP, Souza DO. Guanosine impairs inhibitory avoidance performance in rats. NeuroReport 2000; 11, 2537−2540.

40. Saute JA, Silveira LE, Soares FA, Martini LH, Souza DO, Ganzella M. Amnesic effect of GMP depends on its conversion to guanosine. Neurobiol Learn Mem 2006; 85, 206 - 212.

41. Schmidt AP, Lara DR, Maraschin JF, Perla AS, Souza DO. Guanosine and GMP prevent seizures induced by quinolinic acid in mice. Brain Res 2000; 864, 40 - 43.

42. Schmidt AP, Lara DR, Souza DO. Proposal of a guanine-based purinergic system in the mammalian central nervous system. Pharmacol Ther 2007; 116:401–16.

43. Schmidt AP, Paniz L, Schallenberger C, Böhmer AE, Wofchuk ST, Elisabetsky E, Portela LV, Souza DO. Guanosine prevents thermal hyperalgesia in a rat model of peripheral mononeuropathy. J Pain. 2009; 11:131-41.

44. Schmidt AP, Böhmer AE, Schallenberger C, Antunes C, Tavares RG, Wofchuk ST, Elisabetsky E, Souza DO. Mechanisms involved in the antinociception induced by systemic administration of guanosine in mice. Br J Pharmacol. 2010; 159:1247-63.

45. Sharif NA, Roberts PJ. Regulation of cerebellar L-[3H] glutamate binding: influence of guanine nucleotides and Na+ ions. Biochem Pharmacol 1981; 30:3019–3022.
46. Silva RG, Santos DS, Basso LA, Oses JP, Wofchuk S, Portela LV, Souza DO. Purine nucleoside phosphorylase activity in rat cerebrospinal fluid. Neurochem Res 2004; 29:1831-5.
47. Simon AB, Gorman JM. Advances in the treatment of anxiety: targeting glutamate. NeuroRx 2006; 3,57−68.
48. Soares, F. A., Schmidt, A. P., Farina, M., Frizzo, M. E., Tavares, R. G., Portela, L. V., 2004. Anticonvulsant effect of GMP depends on its conversion to guanosine. Brain Res 1005, 182 - 186.
49. Souza DO, Ramirez G. Effects of guanine nucleotides on kainic acid binding and on adenylate cyclase in chick optic tectum and cerebellum. J Mol Neurosci 1991; 3:39–45.
50. Steckler T, Lavreysen H, Oliveira AM, Aerts N, VanCraenendonck H, Prickaerts J, Megens A, Lesage AS. Effects of mGlu1 receptor blockade on anxiety-related behaviour in the rat lick suppression test. Psychopharmacology 2005; 179, 198−206.
51. Swanson CJ, Bures M, Johnson MP, Linden AM, Monn JA, Schoepp DD. Metabotropic glutamate receptors as novel targets for anxiety and stress disorders. Nat Rev Drug Discov 2005; 4:131−144.
52. Tasca CI, Wofchuk ST, Souza DO, Ramirez G, Rodnight R. Guanine nucleotides inhibit the stimulation of GFAP phosphorylation by glutamate. Neuroreport 1995; 6:249 252.

53. Vanover KE, Suruki, M, Robledo S, Huber M, Wieland S, Lan NC, Gee KW, Wood PL, Carter RB. Positive allosteric modulators of the GABA(A) receptor: differential interaction of benzodiazepines and neuroactive steroids with ethanol. Psychopharmacology 1999; 141:77−82.
54. Vinadé ER, Schmidt AP, Frizzo ME, Portela LV, Soares FA, Schwalm FD. Effects of chronic administered guanosine on behavioral parameters and brain glutamate uptake in rats. J Neurosci Res 2005; 79:248 – 253.
55. Tasca CI, Cardoso LF, Martini LH, Ramirez G, Souza DO. Guanine nucleotides inhibit cAMP accumulation induced by metabotropic glutamate receptor activation. Neurochem Res 1998; 23:183–188.
56. Tasca CI, Cardoso LF, Souza DO. Effects of guanine nucleotides on adenosine and glutamate modulation of cAMP levels in optic tectum slices from chicks. Neurochem Int 1999; 34:213–220.
57. Vinade ER, Izquierdo I, Lara DR, Schmidt AP, Souza DO. Oral administration of guanosine impairs inhibitory avoidance performance in rats and mice. Neurobiol Learn Mem 2004; 81:137−143.
58. Wu LG. Saggau P. Adenosine inhibits evoked synaptic transmission primarily by reducing presynaptic calcium influx in area CA1 of hippocampus, Neuron 1994; 12:1139–1148.
59. Yawo H, Chuhma N. Preferential inhibition of omega-conotoxin-sensitive presynaptic Ca2+ channels by adenosine autoreceptors, Nature 1993; 365:256–258.
60. Zimmermann, H. Ectonucleotidases in the nervous system. Novartis Found Symp 2006a; 276:113−128.

Figures:

Table 1

Table 1 – CSF purines levels 60 min after an i.p. injection of saline		
(uM)	Method I	Method II
ADO	0.04 ± 0.02	0.10 ± 0.04*
INO	0.26 ± 0.07	0.55 ± 0.25*
HIPOX	2.80 ± 0.22	4.00 ± 0.63*
GUO	0.48 ± 0.05	0.52 ± 0.09
XANT	3.77 ± 0.25	2.60 ± 0.17*
UA	1.72 ± 0.17	1.43 ± 0.32

Figure 1

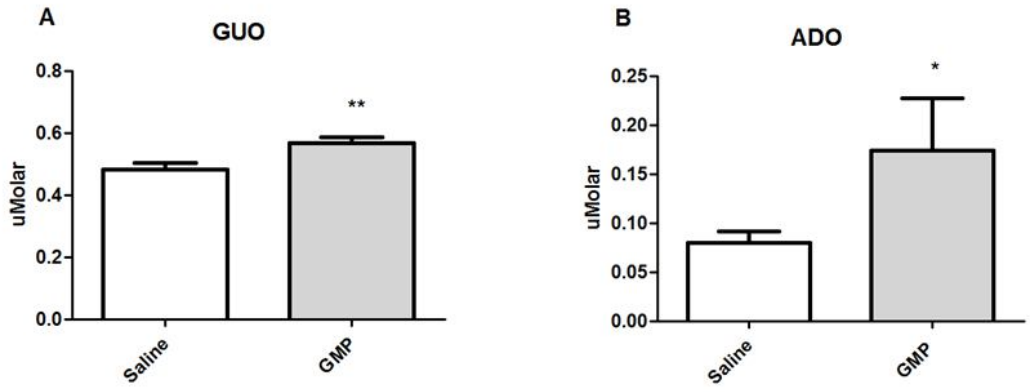


Figure 2

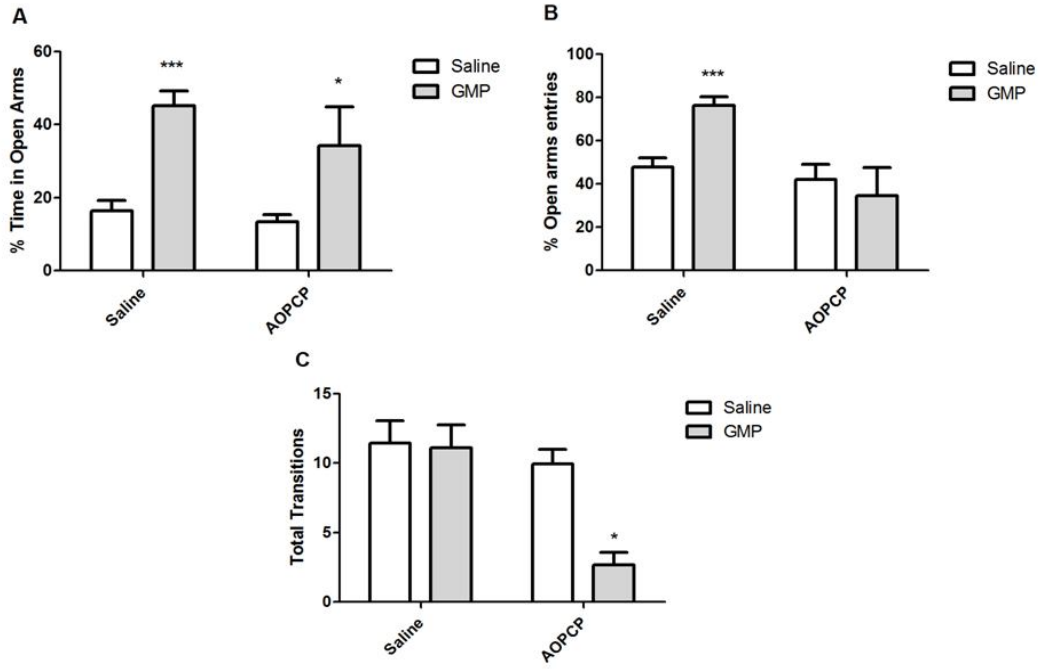


Figure 3

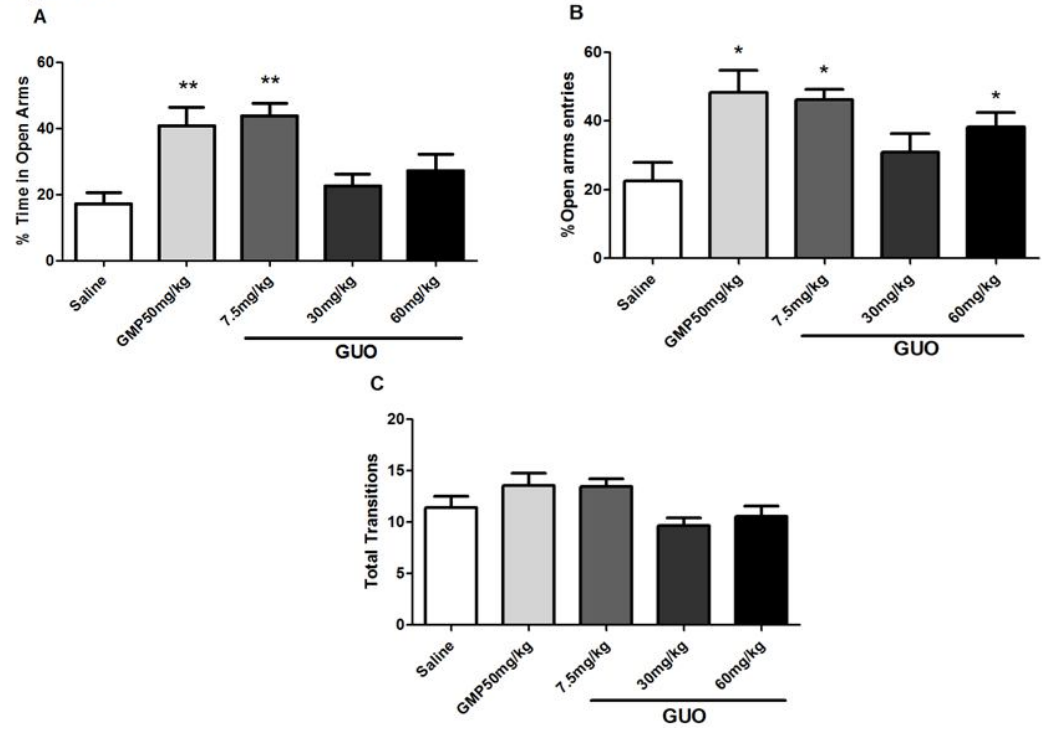


Figure 4

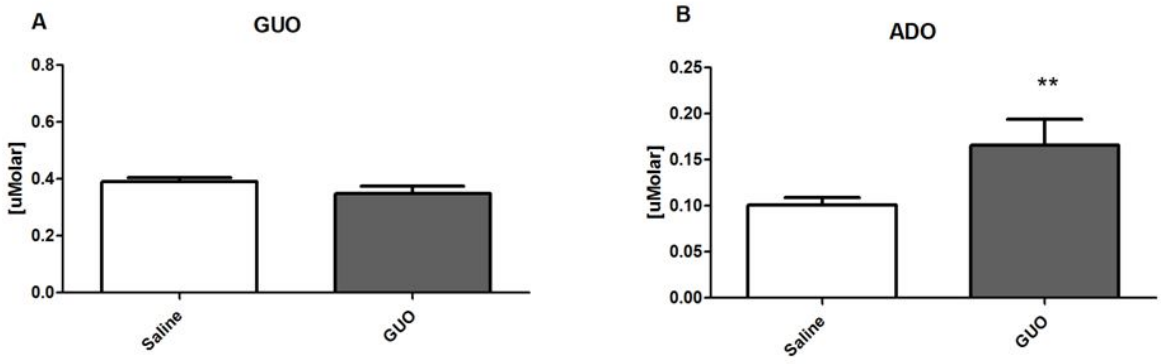


Figure 5

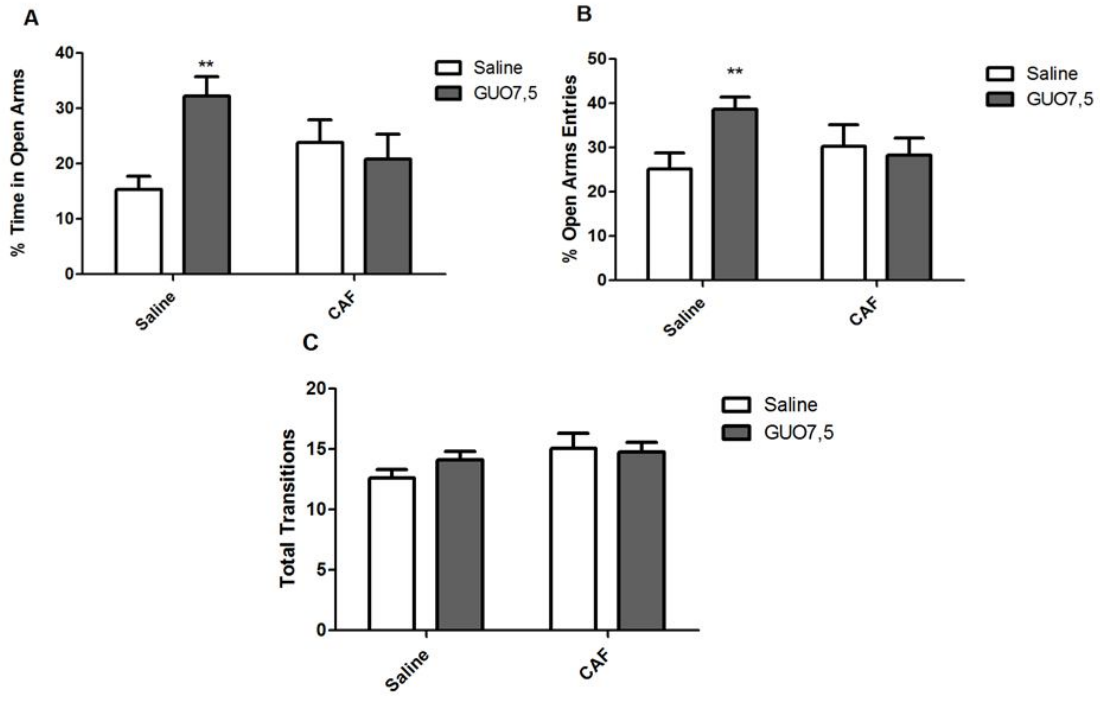
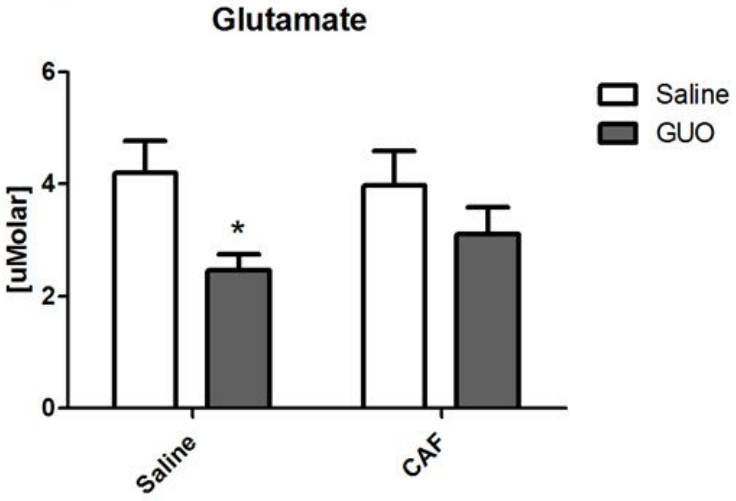


Figure 6



Parte III

DISCUSSÃO

Em estudo realizado pelo nosso grupo, mostramos que a administração sistêmica de GMP foi capaz de exercer efeito típico ansiolítico em ratos, e que tal efeito não foi acompanhado de alterações na concentração de purinas no liquor (Almeida et al., 2010). Inicialmente argumentamos que este efeito poderia ser explicado por alterações anteriores no liquor, já que nossa análise foi realizada após 60 min e já havia sido demonstrado que 30 min após a administração interaperitoneal de GMP os níveis de GUO estavam aumentados (Soares et al., 2004). No entanto, é importante destacar que analisando nossos resultados, grandes variações foram observadas ao compararmos amostras do mesmo grupo.

Levando em consideração que inúmeras enzimas responsáveis pela hidrólise entre os nucleotídeos e nucleosídeos já foram caracterizadas, e que há uma elevada taxa de metabolização do sistema purinérgico no cérebro (Burnstock, G. 2002; Cunha, 2001; Silva et al., 2004; Schmidt et al., 2007; Zimmermann, 2006a), a grande variabilidade expressa em nossos resultados deve estar relacionada a ação destas enzimas no liquor após a coleta.

Diante disto, uma nova metodologia baseada no congelamento imediato das amostras com Nitrogênio Líquido (NL) foi estabelecida. No estudo anterior as amostras eram mantidas em gelo durante cerca de 3 horas (tempo médio de duração do experimento) antes de serem congeladas a -80ºC. Nossos resultados demonstram que o rápido congelamento com NL é capaz de impedir o metabolismo dos nucleosídeos, visto que ADO/INO/HIPOX estão aumentados, e seus metabólitos (XAN/AU) estão diminuídos. Isto nos permite sugerir que esta metodologia é menos variável e torna a análise dos níveis de purinas no liquor mais exata e precisa. Interessantemente, como

exemplo, os níveis de purinas em estudos anteriores variam de aproximadamente 0.1 a 1 μM (Soares et al., 2004; Schmidt et al., 2010; Vinadé et al., 2005).

Assim, após estabelecermos uma metodologia que reduz a variabilidade na dosagem de purinas no liquor, nossa primeira estratégia foi re-avaliar o efeito de uma administração intraperitoneal de GMP nos níveis de purinas no liquor. Nossos resultados mostraram que os níveis de GUO, assim como os níveis de adenosina (ADO) aumentaram após a administração sistêmica de GMP. No entanto, é importante salientar que os níveis de GMP não foram detectados.

Diante dos aumentos dos níveis de GUO no liquor, avaliamos se o mecanismo de ação da administração sistêmica de GMP seria central e dependente da sua conversão a GUO, utilizando uma metodologia semelhante a utilizada em estudos anteriores (Saute et al., 2006; Soares et al., 2004). Ao realizarmos um pré-tratamento com um inibidor da enzima ecto-5'-nucleotidase AOPCP (α, ß-methyleneadenosine 5'-diphosphate) verificamos que administração i.c.v. de GMP induz efeito típico ansiolítico. Contudo, inesperadamente, quando os animais foram tratados com AOPCP e GMP houve um prejuízo significativo na locomoção dos animais. Como em outras tarefas comportamentais, a atividade locomotora dos animais deve ser observada, inclusive nos parâmetros utilizados para avaliar comportamentos típicos ansiolíticos em ratos. Sendo assim, ficou comprometida a utilização deste protocolo para investigar se o efeito ansiolítico da administração central de GMP é devido a sua conversão a GUO, dado o efeito observado na atividade locomotora pela administração do AOPCP.

Após estes resultados, buscamos investigar se os efeitos de uma adminsitração intraperitoneal aguda de diferentes doses de GUO reproduziriam os efeitos do tratamento com GMP. Conforme demonstramos, o tratamento com GUO 7.5 mg/kg

foi capaz de promover um efeito típico ansiolítico similar ao do GMP no labirinto em cruz elevado. Surpreendentemente, ambos os tratamentos aumentaram os níveis de ADO, e apenas o tratamento com GMP aumentou os níveis de GUO no liquor. Assim, esses dados nos levaram a hipotetizar que o efeito ansiolítico observado com a administração de GMP e GUO podem ter o envolvimento do sistema adenosinérgico.

De acordo com alguns estudos do nosso grupo, o sistema adenosinérgico não parece estar envolvidos em alguns efeitos das PDGs (Lara et al., 2001; Roesler et al., 2000; Vinade et al., 2004). Entretanto, já foi observado por Ciccarelli e colaboradores que a adição de GUO em cultura de astrócitos aumenta a liberação de ADO, e que tal efeito poderia ser responsável pelos efeitos tróficos promovidos pela GUO. (Ciccarelli et al., 2000). Além disso, outros resultados do nosso grupo já mostraram que os efeitos antinociceptivos da GUO em camundongos parecem estar relacionados com a modulação dos receptores de adenosina A1 e A2A (Schmidt et al., 2010).

Adicionalmente, o sistema adenosinérgico parece estar intimamente relacionado com a modulação dos transtornos de ansiedade. Sabe-se que a inibição da transmissão sináptica mediada pela ADO, diminui a liberação de glutamato no sistema nervoso central via ativação dos receptores de adenosina A1 (Ambrósio, et al., 1997; Wu and Saggau, 1994; Yawo and Chuhma, 1993). Como este efeito parece ser uma estratégia efetiva para futuros fármacos com ação ansiolítica (Swanson et al., 2005; Simon and Gorman, 2006; Steckler et al., 2005), estudos já demonstraram que agonistas de receptores A1 possuem efeitos ansiolíticos (Florio et al., 1998, Jain et al., 1995). Ainda, Johansson e colaboradores mostraram que camundongos que tiveram os genes para receptores A1 deletados geneticamente apresentaram aumento da ansiedade em comportamentos relacionados à ansiedade (Johansson et al., 2001).

Afim de investigar o envolvimento do sistema adenosinérgico no efeito típico ansiolítico obtido com a administração sistêmica de GUO, investigamos o efeito de um pré-tratamento com cafeína (CAF, um antagonista inespecífico não-seletivo dos receptores de ADO). Nossos resultados demonstraram que o pré-tratamento com CAF aboliu o efeito ansiolítico da GUO, indicando que o efeito ansiolítico da GUO envolve o sistema adenosinérgico.

Muitas evidências sugerem que tanto a ADO, como a GUO são capazes de modular a neurotransmissão glutamatérgica. Nosso grupo já mostrou que a GUO aumenta a captação astrocitária de glutamato quando há uma hiper ativação deste sistema (Frizzo et al., 2002). Além disso, nossos resultados demonstram que a administração de GUO diminuiu os níveis de glutamato, sem qualquer alteração na captação de glutamato. Assim, estes dados reforçam a hipótese anterior do nosso grupo de que o aumento da captação de glutamato promovido pela GUO ocorre apenas em condições relacionadas a excitotoxicidade, condições essas que não ocorrem em comportamentos relacionados à ansiedade utilizados neste estudo. Ainda, diante dos nossos resultados podemos especular que a GUO poderia modular o tônus glutamatérgico indiretamente pelo aumento dos níveis de ADO.

Sendo assim, podemos observar o potencial efeito ansiolítico da GUO envolve tanto o sistema adenosinérgico, como a modulação dos níveis de glutamato no liquor. Levando em consideração os resultados apresentados nesta dissertação novas evidências do mecanismo de ação que ajudam a explicar o efeito ansiolítico promovido pelas PDGs foram evidenciados.

CONCLUSÕES

Os resultados apresentados na presente dissertação possibilitaram melhorar a compreensão sobre o mecanismo de ação das PDGs. Como importante contribuição, mostramos que os sistemas adenosinérgico e glutamatérgico estão envolvidos no potencial efeito ansiolítico promovido pela administração das PDGs. Finalmente, esse estudo estende o potencial das PDGs como estratégias para o desenvolvimento de novos fármacos, com efeitos colaterais reduzidos, para o tratamento de transtornos psiquiátricos.

REFERÊNCIAS BIBLIOGRÁFICAS

1. Almeida RF, Cereser VH, Faraco RB, Böhmer AE, Souza DO, Ganzella M. Systemic administration of GMP induces anxiolytic-like behavior in rats. Pharmacology Biochemistry and Behavior, 2010; 96(3), 306 -311.
2. Ambrósio AF, Malva JO, Carvalho AP, Carvalho CM. Inhibition of N-, P/Q- and other types of Ca2+ channels in rat hippocampal nerve terminals by the adenosine A1 receptor, Eur. J. Pharmacol 1997; 340:301–310.
3. Arriza JL, Eliasof S, Kavanaugh MP, Amara SG., 1997. Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance. Proc Natl Acad Sci USA 1994; 4155 - 4160.
4. Baron BM, Dudley MW, McCarty DR, Miller FP, Reynolds IJ, Schmidt CJ. Guanine nucleotides are competitive inhibitors of N-Methyl-D-Aspartate at its receptor site both in vitro and in vivo. Pharmacology and Experimental Therapeutics 1989; 250, 162 - 169.
5. Bergink V, van Megen HJ, Westenberg HG. Glutamate and anxiety. Eur Neuropsychopharmacol 2004; 14, 175 – 183.
6. Burgos JS, Barat A, Souza DO, Ramírez G. Guanine nucleotides protect against kainate toxicity in an ex vivo chick retinal preparation. FEBS Letters 1998; 430, 176 - 180.
7. Burnstock, G. Purinergic signaling: an overview. Novartis Found Symp 2006b; 276:26−48.
8. Chao XD, Fei F, Fei Z. The role of excitatory amino acid transporters in cerebral ischemia. Neurochem Res. 2010; 35, 1224-1230.
9. Chojnacka-Wojcik E, Klodzinska A, Pilc A. Glutamate receptor ligands as anxiolytics. Curr Opin Investig Drugs 2001; 2, 1112 − 1119.

10. Ciccarelli R, Di Iorio P, Giuliani P, D'Alimonte I, Ballerini P, Caciagli F. Rat cultured astrocytes release guanine-based purines in basal conditions and after hypoxia/hypoglycemia. Glia 1999; 25, 93 - 98.
11. Ciccarelli R, Ballerini P, Sabatino G, Rathbone MP, D'Onofrio M., Caciagli F, Di Iorio P. Involvement of astrocytes in purine-mediated reparative processes in the brain. International Journal of Developmental Neuroscience 2001; 19, 395 - 414.
12. Corbett R, Dunn RW. Effects of 5, 7 dichlorokynurenic acid on conflict, social interaction and plus maze behaviors. Neuropharmacology 1993; 32, 461 - 466.
13. Cotman CW, Kahle JS, Miller SE, Ulas J, Bridges RJ. Excitatory aminoacid neurotransmition. Psychopharmacology: The fourth generation of progress 1995; 7, 75 – 85.
14. Cryan JF, Kelly PH, Neijt HC, Sansig G, Flor PJ, van Der Putten H. Antidepressant and anxiolytic-like effects in mice lacking the group III metabotropic glutamate receptor mGluR7. European Journal of Neuroscience 2003; 11, 2409 - 2417.
15. Cunha, RA.Regulation of the ecto-nucleotidase pathway in rat hippocampal nerve terminals. Neurochem Res 2001; 26:979–991.
16. Danbolt NC. Glutamate uptake. Prog Neurobiol 2001; 65:1-105
17. Duan S, Anderson CM, Stein BA, Swanson RA. Glutamate induces rapid upregulation of astrocyte glutamate transport and cell-surface expression of GLAST. J Neurosci 1999; 19, 10193-10200.
18. Dunn RW, Corbett R, Fielding S. Effects of 5-HT1A receptor agonists and NMDA receptor antagonists in the social interaction test and the elevated plus maze. European Journal of Pharmacology 1989; 169, 1- 10.

19. Eulenburg V, Gomeza J. Neurotransmitter transporters expressed in glial cells as regulators of synapse function. Brain Res Rev. 2010; 63:103-112.
20. Florio C, Prezioso A, Papaioannou A, Vertua R. Adenosine A1 receptors modulate anxiety in CD1 mice. Psychopharmacology 1998; 136:311-319.
21. Frizzo ME, Lara DR, Dahm KCS, Prokopiuk AS, Swanson RA, Souza DO. Activation of glutamate uptake by guanosine in primary astrocyte cultures. Neuroreport 2001; 12, 879 - 881.
22. Frizzo ME, Soares AF, Dall'Onder LP, Lara DR, Swanson RA, Souza DO. Extracellular conversion of guanine-based purines to guanosine specifically enhances astrocyte glutamate uptake. Brain Research 2003; 972, 84 – 89.
23. Gudermann T, Schorneberg T, Schultz G. Functional and structural complexity of signal transduction via G-protein-coupled receptors. Annual Review of Neuroscience 1997; 20, 399 – 427.
24. Hamner MB, Robert S, Frueh BC. Treatment-resistant posttraumatic stress disorder: strategies for intervention. CNS Spectr. 2004; 9, 740 - 752.
25. Hoffman EJ, Mathew SJ. Anxiety Disorders: A Comprehensive Review of Pharmacotherapies. Mount Sinai Journal of Medicine 2008; 75, 248 – 262.
26. Izquierdo I, Bevilaqua LR, Rossato JI, Bonini JS, Medina JH, Cammarota M. Different molecular cascades in different sites of the brain control memory consolidation. Trends in Neurosciences 2006; 29, 496 − 505.
27. Jain N, Kemp N, Adeyemo O, Buchanan P, Stone TW Anxiolytic activity of adenosine receptor activation in mice. Br J Pharmacol 1995; 116:2127-2133.
28. Johansson B, Halldner L, Dunwiddie TV, Masino SA, Poelchen W, Giménez Llort L, Escorihuela RM, FernándezTeruel A, Wiesenfeld-Hallin Z, Xu XJ, Hrdemark A, Betsholtz C, Herlenius E, Fredholm BB. Hyperalgesia, anxiety,

and decreased hypoxic neuroprotection in mice lackingthe adenosine A1 receptor. Proc Natl Acad Sci 2001; 98:9407-9412.

29. Kapus GL, Gacsályi I, Vegh M, Kompagne H, Hegedus E, Leveleki C, Hársing LG, Barkóczy J, Bilkei-Gorzó A, Lévay G. Antagonism of AMPA receptors produces anxiolytic-like behavior in rodents: effects of GYKI 52466 and its novel analogues. Psychopharmacology 2008; 198, 231 - 241.
30. Kehne JH, McCloskey TC, Baron BM, Chi EM, Harrison BL, Whitten JP. NMDA receptor complex antagonists have potential anxiolytic effects as measured with separation-induced ultrasonic vocalizations. European Journal of Pharmacology 1991; 193, 283 − 292.
31. Kotlinska J, Liljequist S. The putative AMPA receptor antagonist, LY326325, produces anxiolytic-like effects without altering locomotor activity in rats. Pharmacol Biochem Behav. 1998; 60, 119 -124.
32. Lara DR, Schmidt AP, Frizzo ME, Burgos JS, Ramirez G, Souza DO. Effect of orally administered guanosine on seizures and death induced by glutamatergic agents. Brain Research 2001; 912, 176 - 180.
33. Lépine JP.The epidemiology of anxietydisorders: prevalence and societal costs. J Clin Psychiatry. 2002; 63, 4 - 8.
34. Liberzon I, Phan KL, Khan S, Abelson JL. Role of GABA-A receptors in anxiety: Evidence from animal models, clinical psychopharmacology, and neuroimaging studies. Current Neuropharmacology. 2003; 1, 267-283.
35. Lipton SA, Rosenberg PA. Excitatory amino acids as a final common pathway for neurologic disorders. The New England Journal of Medicine 1994; 330, 613 − 622.

36. Machado-Vieira R, Manji HK, Zarate CA. The role of the tripartite glutamatergic synapse in the pathophysiology and therapeutics of mood disorders. Neuroscientist. 2009; 15, (5):525 - 39.
37. Malcon C, Achaval M, Komlos F, Partata W, Sauressig M, Ramirez G, Souza DO. GMP protects against quinolinic acid-induced loss of NADPH-diaphorase-positive cells in the rat striatum. Neuroscience Letters 1997; 225, 145 – 148.
38. Maragakis NJ, Rothstein JD. Glutamate transporters: animal models to neurologic disease. Neurobiol Dis. 2004; 15, 461-473.
39. Maragakis NJ, Rothstein JD. Mechanisms of disease: astrocytes in neurodegenerative disease. Natural Clinical Practice Neurology. 2006; 2, 679 - 689.
40. Meldrum BS. The role of glutamate in epilepsy and other CNS disorders. Neurology 1994; 44, 14 − 23.
41. Moussa CE, Rae C, Bubb WA, Griffin JL, Deters NA, Balcar VJ. Inhibitors of glutamate transport modulate distinct patterns in brain metabolism. J Neurosc Res. 2007; 85, 342 - 350.
42. Nutt DJ, Malizia AL. New insights into the role of the GABA(A)-benzodiazepine receptor in psychiatric disorder. The British Journal of Psychiatry 2001; 179, 390 - 396.
43. Oliveira DL, Horn JF, Rodrigues JM, Frizzo ME, Moriguchi E, Souza DO. Quinolinic acid promotes seizures and decreases glutamate uptake in young rats: reversal by orally administered guanosine. Brain Research 2004; 1018, 48 - 54.
44. Ozawa S, Kamiya H, Tsuzuki K. Glutamate receptor in the mammalian central nervous system. Progress in Neurobiology 1998; 54, 581 – 618.

45. Paas Y, Thiery AD, Changeux JP, Medevielle F, Teichberg VI. Identification of an extracellular motif involved in the binding of guanine nucleotides by a glutamate receptor. The EMBO Journal 1996; 15, 1548 – 1556.
46. Palucha A, Pilc A. Metabotropic glutamate receptor ligands as possible anxiolytic and antidepressant drugs. Pharmacology & Therapeutics 2007; 115, 116 – 147.
47. Paz MM, Ramos M, Ramirez G, Souza DO. Differential effects of guanine nucleotides on kainic acid binding and on adenylate cyclase activity in chick optic tectum. FEBS Letters 1994; 355, 205 - 208.
48. Plaznik A, Palejko W, Nazar M, Jessa M. Effects of antagonists at the NMDA receptor complex in two models of anxiety. European Neuropsychopharmacology 1994; 4, 503 - 512.
49. Rathbone MP, Middlemiss PJ, Gysbers JW, Andrew C, Herman MAR, Reed JK, Ciccarelli R, Di Iorio P, Caciagli F. Trophic effects of purines in neurons and glial cells. Progress in Neurobiology 1999; 59, 663 – 690.
50. Regner A, Ramírez G, Belló-Klein A, Souza DO. Effects of guanine nucleotides on glutamate-induced chemiluminescence in rat hippocampal slices submitted to hypoxia. Neurochemical Research. 1998 ; 23, 519 - 524.
51. Robinson MB. Acute regulation of sodium-dependent glutamate transporters: a focus on constitutive and regulated trafficking. Handb Exp Pharmacol. 2006; 175:251-275.
52. Roesler R, Vianna MR, Lara DR, Izquierdo I. Schmidt AP, Souza DO. Guanosine impairs inhibitory avoidance performance in rats. NeuroReport 2000; 11, 2537−2540.

53. Saute JA, Silveira LE, Soares FA, Martini LH, Souza DO, Ganzella M. Amnesic effect of GMP depends on its conversion to guanosine. Neurobiology of Learn and Memory 2006; 85, 206 - 212.

54. Swanson CJ, Bures M, Johnson MP, Linden AM, Monn JA, Schoepp DD. Metabotropic glutamate receptors as novel targets for anxiety and stress disorders. Nat Rev Drug Discov 2005; 4:131−144.

55. Schmidt AP, Lara DR, Maraschin JF, Perla AS, Souza DO. Guanosine and GMP prevent seizures induced by quinolinic acid in mice. Brain Research. 2000; 864, 40 - 43.

56. Schmidt AP, Ávila TT, Souza DO. Intracerebroventricular guanine-based purines protect against seizures induced by quinolinic acid in mice. Neurochemical Research. 2005; 30, 69 - 73.

57. Schmidt AP, Lara DR, Souza DO. Proposal of a guanine-based purinergic system in the mammalian central nervous system. Pharmacology & Therapeutics. 2007; 116, 401 – 416.

58. Schmidt AP, Böhmer AE, Leke R, Schallenberger C, Antunes C, Pereira MS, Wofchuk ST, Elisabetsky E, Souza DO. Antinociceptive effects of intracerebroventricular administration of guanine-based purines in mice: evidences for the mechanism of action. Brain Research. 2008; 1234, 50 - 58.

59. Schmidt AP, Böhmer AE, Schallenberger C, Antunes C, Tavares RG, Wofchuk ST, Elisabetsky E, Souza DO. Mechanisms involved in the antinociception induced by systemic administration of guanosine in mice. Br J Pharmacol. 2010; 159:1247-63.

60. Sheldon AL, Robinson MB. The role of glutamate transporters in neurodegenerative diseases and potential opportunities for intervention. Neurochemistry International. 2007; 51, 333 - 355.
61. Segovia G, Porras A, Del Arco A, Mora F. Glutamatergic neurotransmission in aging: a critical perspective. Mechanisms of Ageing and Development. 2001; 122, 1 - 29.
62. Silva RG, Santos DS, Basso LA, Oses JP, Wofchuk S, Portela LV, Souza DO. Purine nucleoside phosphorylase activity in rat cerebrospinal fluid. Neurochem Res 2004; 29:1831-5.
63. Simon AB, Gorman JM. Advances in the Treatment of Anxiety: Targeting Glutamate. NeuroRx. 2006 Jan;3(1):57-68.
64. Soares FA, Schmidt AP, Farina M, Frizzo ME, Tavares RG, Portela LV. Anticonvulsant effect of GMP depends on its conversion to guanosine. Brain Research. 2004; 1005, 182 - 186.
65. Souza DO, Frizzo ME, Lara DR. Glutamate uptake enhanced by guanosine in vitro: possible neuroprotective mechanism against hypoxia/hypoglycemia injury. Drug Development Research. 2000; 50, 115.
66. Spooren W, Gasparini F. mGlu5 receptor antagonists: a novel class of anxiolytics? Drug News & Perspectives. 2004; 17, 251 − 257.
67. Steckler T, Lavreysen H, Oliveira AM, Aerts N, VanCraenendonck H, Prickaerts J, Megens A, Lesage AS. Effects of mGlu1 receptor blockade on anxiety-related behaviour in the rat lick suppression test. Psychopharmacology 2005; 179, 198−206.
68. Swanson RA, Ying W, Kauppinen TM. Astrocyte influences on ischemic neuronal death. Curr. Mol. Med. 2004; 4:193-205.

69. Tasca CI, Wofchuk ST, Souza DO, Ramirez G, Rodnight R. Guanine nucleotides inhibit the stimulation of GFAP phosphorylation by glutamate, NeuroReport. 1995; 6, 249 – 252.

70. Tasca CI, Cardoso LF, Martini LH, Ramirez G, Souza DO. Guanine nucleotides inhibit cAMP accumulation induced by metabotropic glutamate receptor activation. Neurochemical Research. 1998; 23, 183 – 188.

71. Tatarczynska E, Klodzinska A, Chojnacka-Wojcik E, Palucha A, Gasparini F, Kuhn R. Potential anxiolytic- and antidepressant-like effects of MPEP, a potent, selective and systemically active mGlu5 receptor antagonist. British Journal of Pharmacology. 2001a; 132, 1423 − 1430.

72. Tatarczynska E, Klodzinska A, Kroczka B, Chojnacka-Wojcik E, Pilc A. The antianxiety-like effects of antagonists of group I and agonists of group II and III metabotropic glutamate receptors after intrahippocampal administration. Psychopharmacology. 2001b; 158, 94 − 99.

73. Tziugouris AV, Wadiche JI. Glutamate transporters: confining runaway excitation by shaping synaptic transmission. Nature. 2007; 8:935-947.

74. Valença AM. Transtornos de ansiedade: Epidemiologia e impacto social. SNC em foco. 2005; 1, 3 - 9.

75. van Ameringen M, Mancini C, Pipe B, Bennett M. Optimizing treatment in social phobia: a review of treatment resistance. CNS Spectr. 2004; 9:753-762.

76. Vinadé ER, Schmidt AP, Frizzo ME, Izquierdo I, Elizabetsky E, Souza DO. Chronically administered guanosine is anticonvulsant, amnesic and anxiolytic in mice. Brain Research. 2003; 977, 97 - 102.

77. Vinade ER, Izquierdo I, Lara DR, Schmidt AP, Souza DO. Oral administration of guanosine impairs inhibitory avoidance performance in rats and mice. Neurobiol Learn Mem 2004; 81:137−143.
78. Vinadé ER, Schmidt AP, Frizzo ME, Portela LV, Soares FA, Schwalm FD. Effects of chronic administered guanosine on behavioral parameters and brain glutamate uptake in rats. Journal of Neuroscience Research. 2005; 79, 248 – 253.
79. Winslow JT, Insel TR, Trullas R, Skolnick P. Rat pup isolation calls are reduced by functional antagonists of the NMDA receptor complex. European Journal of Pharmacology. 1990; 190, 11 - 21.
80. Wu LG. Saggau P. Adenosine inhibits evoked synaptic transmission primarily by reducing presynaptic calcium influx in area CA1 of hippocampus, Neuron 1994; 12:1139–1148.
81. Yawo H, Chuhma N. Preferential inhibition of omega-conotoxin-sensitive presynaptic Ca2+ channels by adenosine autoreceptors, Nature 1993; 365:256–258.
82. Zimmermann, H. Ectonucleotidases in the nervous system. Novartis Found Symp 2006a; 276:113−128.