Uliginosin B, a Possible New Analgesic Drug, Acts by Modulating the Adenosinergic System

Eveline Dischkaln Stolz,1 Paola Fontoura da Costa,1 Liciane Fernandes Medeiros,2 Andressa Souza,2 Ana Maria Oliveira Battastini,3 Gilsane Lino von Poser,1 Carla Bonan,4 Iraci L. S. Torres,2 and Stela Maris Kuze Rates1

1Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, 90610-000 Porto Alegre, Brazil
2Laboratório de Farmacologia da Dor e Neuromodulação: Investigações Pré-Clinicas, Departamento de Farmacologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, 90046-900 Porto Alegre, Brazil
3Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, 90035-003 Porto Alegre, Brazil
4Laboratório de Neuroquímica e Psicofarmacologia, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, 90619-900 Porto Alegre, Brazil

Correspondence should be addressed to Stela Maris Kuze Rates; stela.rates@ufrgs.br

Received 28 January 2016; Accepted 21 February 2016

Academic Editor: Vincenzo De Feo

Copyright © 2016 Eveline Dischkaln Stolz et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Uliginosin B (ULI) is a natural acylphloroglucinol that has been proposed as a new molecular scaffold for developing analgesic and antidepressant drugs. Its effects seem to be due to its ability to increase monoamines in the synaptic cleft by inhibiting their neuronal uptake without binding to their respective transporters, but its exact mode of action is still unknown. Considering the importance of the purinergic system to pain transmission and its modulation by monoamines availability, the aim of this study was to investigate the involvement of adenosinergic signaling in antinociceptive effect of uliginosin B. The selective adenosine A1 receptor antagonist DPCPX and the selective A2A antagonist ZM 241385 prevented the effect of ULI in the hot-plate test in mice. Pretreatment with inhibitors of adenosine reuptake (dipyridamole) or adenosine deaminase (EHNA) did not affect the ULI effect. On the other hand, its effect was completely prevented by an inhibitor of ecto-5'-nucleotidase (AMPCP). This finding was confirmed ex vivo, whereby ULI treatment increased AMP and ATP hydrolysis in spinal cord and cerebral cortex synaptosomes, respectively. Altogether, these data indicate that activation of A1 and A2A receptors and the modulation of ecto-5'-nucleotidase activity contribute to the antinociceptive effect of ULI.

1. Introduction

Uliginosin B (ULI) is a dimeric acylphloroglucinol consisting of filicinic acid and phloroglucinol moieties, which occurs in Hypericum species native to South America [1]. This molecular pattern has been proposed as a prototype to develop analgesic and antidepressant drugs [2–5].

Preclinical studies suggested that ULI has antidepressant properties, which seems to be due to its ability to increase monoamines availability in the synaptic cleft by inhibiting their neuronal uptake [2]. Nevertheless, ULI does not bind to the monoamine sites on neuronal transporters, which indicates that it acts differently from the classical antidepressants [2]. It is noteworthy that ULI deserves attention as a drug potentially useful to reduce the dose of morphine in clinical practice [6]. Its antinociceptive effect involves the activation of monoaminergic, glutamatergic, and opioid receptors, apparently without binding to these receptors [2, 3, 5]. Therefore, other molecular targets for ULI might be considered.

The relationship between purinergic system/nociceptive pathways has been reported [7]; numerous studies described the interaction between purinergic, monoaminergic, and opioid pathways [8–14].
Adenosine triphosphate (ATP) stimulates cellular excitability, augments the release of excitatory amino acids, initiates a nociceptive response, and can lead to apoptosis [15,16]. ATP released from cells into the extracellular space has a short half-life in the extracellular milieu since it is rapidly degraded to adenosine diphosphate (ADP), adenosine monophosphate (AMP), and adenosine by ectonucleotidase pathway, which includes the E-NTPDase family (ectonucleoside triphosphate diphosphohydrolase) and ecto-5’-nucleotidase (for review see Robson et al. [17] and Zylka [18]). These enzymes control the availability of ligands (ATP, ADP, AMP, and adenosine) to activate purinoceptors, as well as the duration of receptor activation. In addition, these enzymes may provide a protective function by maintaining extracellular ATP/ADP and adenosine levels within physiological concentrations (for review see Burnstock [19]). Adenosine levels are also controlled by deamination to inosine through adenosine deaminase (ADA), cell release, and reuptake through nucleoside transporters (NTs) in bidirectional equilibrative processes driven by chemical gradients and unidirectional concentrative processes driven by sodium electrochemical gradients [20,21]. The activation of adenosine receptors appears to be involved in the modulation of nociceptive and inflammatory pathways [7]. These effects depend on the availability of adenosine in the synaptic cleft, as well as intensity and modality of the stimulus [11].

Interestingly, several drugs that increase monoamine availability or act through the activation of opioid receptors present antinociceptive effect mediated by activation of adenosine receptors [12–14].

In view of these observations, the aim of this study was to investigate the involvement of purinergic pathway in the antinociceptive effect of ULI, including the effect of ULI on adenosine metabolism.

2. Material and Methods

2.1. Uliginosin B Obtention. ULI (Figure 1(a)) was obtained according to Stolz and coworkers [3] from n-hexane extract of the aerial parts (all sections above ground) of Hypericum polyanthemum Klotzsch ex Reichardt (Hypericaceae) (Figure 1(b)), harvested in Caçapava do Sul, Brazil (voucher specimen ICN175915). Plant collection was authorized by the Conselho de Gestão do Patrimônio Genético and Instituto Brasileiro do Meio Ambiente (number 003/2008, Protocol 02000.001717/2008-60).

The purity (96%) of uliginosin B was confirmed through HPLC analysis coupled to an ultraviolet detector [1,22] and its structure was characterized by 1H and 13C NMR spectra [23]. It was stored at −20°C, protected from light and moisture until use. Immediately before biological testing, it was suspended in saline containing 2% polysorbate 80. Unpublished studies by us demonstrated that, in these storage conditions, ULI has good stability and remains unaltered for approximately 2 years.

2.2. Animals. Adult male CFI mice (25–35 g) were used for in vivo and ex vivo experiments. Animals were housed under a 12-hour light/dark cycle (lights on at 7:00 am) at constant temperature (23 ± 1°C) with free access to standard certified rodent diet and tap water. All experiments were approved by a local Ethics Committee of Animal Use (UFRGS: 21060/2011) and were in compliance with Brazilian law [24–26] and conformed to the Laboratory Guide for the Care and Use of Animals [27]. Animal handling and all experiments were performed in accordance with international guidelines for animal welfare and measures were taken to minimize animal pain and discomfort.

2.3. Behavioral Experiments. Pain sensitivity was assessed by the hot-plate test as described elsewhere [3]. First, each animal freely explored the nonfunctioning hot-plate apparatus for 60 s. Then, the animal returned to its home-cage and the apparatus was turned on and stabilized at 55 ± 1°C. Mice baseline responsiveness was determined by recording the time elapsed until the animal licked one of its hind paws or jumped. Mice that presented a baseline reaction of more than 20 s were not used. Immediately, the animals received one of the following compounds: adenosine A1 receptor antagonist: 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) 0.1 mg/kg (0.01 mg/mL, i.p.); adenosine A2A receptors antagonists: 4-(2-(7-amino-2-(furan-2-yl) triazolo[1,5-a][1,3,5]triazin-5-ylamino)ethyl)phenol (ZM 241385) 3 mg/kg (0.3 mg/mL, i.p.); inhibitor of adenosine deaminase: erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) 5 mg/kg (0.5 mg/mL, i.p.); adenosine reuptake inhibitor: dipryridamole (DIP) 30 mg/kg (3 mg/mL, i.p.); ecto-5’-nucleotidase inhibitor: alpha-beta-methylene adenosine 5’-diphosphate (AMPCP) 2 mg/kg (0.2 mg/mL, i.p.). The doses of each tested drug were chosen based on the literature, and lack of antinociceptive effect in the hot-plate test was confirmed in our laboratory [28–31]. After 15 min, the animals were treated with ULI 15 mg/kg (1.5 mg/mL, i.p.) or vehicle (saline plus 2% polysorbate 80; 1 mL/100 g, i.p.) and reexposed to the hot-plate (55 ± 1°C) 30 min later. A maximum latency time of 40 s was imposed (cut-off). The results are expressed as percentages of maximal possible analgesic effect (% MPE) using the following formula:

\[
\% \text{ MPE} = \frac{(\text{post-drug latency} - \text{pre-drug latency})}{(\text{cut-off latency} - \text{pre-drug latency})} \times 100.
\]

2.4. NTPDase and Ecto-5’-nucleotidase Activity

2.4.1. Synaptosomal Preparation. The mice were divided into three groups: handled only (sham), treated with 15 mg/kg ULI (1.5 mg/mL, i.p.), or vehicle (saline plus 2% polysorbate 80, i.p.). After 30 min the animals were killed and the spinal cord and cerebral cortex were removed. The tissues were prepared according to Rozisky et al. [32] and synaptosomes were isolated as described by Nagy and Delgado-Escueta [33]. Protein concentration was determined by the Coomassie blue method [34] using bovine serum albumin as a standard.
2.4.2. Determination of NTPDases and Ecto-5′-nucleotidase Activity. The ATP, ADP, and AMP hydrolysis was performed as described previously [32, 35]. The synaptosomal fraction (10–20 μg protein) was preincubated for 10 min at 37°C in 100 μL of incubation medium containing 45 mM Tris-HCl buffer (pH 8), 0.1 mM EDTA, 1.5 mM CaCl₂, 5 mM KCl, 10 mM glucose, and 225 mM sucrose for ATP and ADP hydrolysis. For AMP hydrolysis the samples were incubated in 80 μL of incubation medium containing 0.1 M Tris-HCl (pH 7), 10 mM MgCl₂, and 0.15 M sucrose. The reactions were initiated by the addition of 1 mM ATP, ADP, or AMP and stopped by the addition of 200 μL of 10% trichloroacetic acid. Finally, 100 μL samples were taken for the assay of released inorganic phosphate (Pi) [36]. The enzyme activities were expressed as nmol of inorganic phosphate released per minute per milligram of protein (nmol Pi·min⁻¹·mg⁻¹ protein).

2.5. Statistical Analysis. The results were evaluated by one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test using the Sigma Stat software, version 2.03 (Jandel Scientific Corporation). All results were expressed as mean ± standard error of the mean (SEM).

3. Results

The influence of selective A₁ and A₂A receptor antagonist pretreatments on the ULI effect in the hot-plate test is depicted in Figure 2. One-way ANOVA revealed a significant antinociceptive effect of ULI (Figure 2(a): F(3,35) = 25.611, p < 0.001; Figure 2(b): F(3,35) = 19.555, p < 0.001), which was prevented by pretreatment with the adenosine A₁ receptor antagonist DPCPX (p < 0.001) and the adenosine A₂A receptor antagonist ZM 241385 (p < 0.001).

The data depicted in Figure 3 show the effect of adenosine metabolism on the antinociceptive effect of ULI. Pretreatment with inhibitor of adenosine deaminase (EHNA) or nucleoside transporter inhibitor (dipryridamole) did not affect the ULI nociceptive response. One-way ANOVA revealed a significant effect in the group treated with ULI and ULI plus EHNA or ULI plus dipryridamole in relation to the control groups (Figure 3(a): F(3,35) = 17.819, p < 0.001; Figure 3(b): F(3,44) = 19.248, p < 0.001). Pretreatment with ecto-5′-nucleotidase inhibitor (AMPCP) prevented the ULI antinociceptive effect on the hot-plate test. One-way ANOVA revealed a significant effect only in the group treated with ULI (Figure 3(c): F(3,35) = 12.981, p < 0.001), which was prevented by the AMPCP pretreatment (p < 0.001).

The activities of NTPDases and ecto-5′-nucleotidase were assessed ex vivo after acute treatment of mice with ULI (15 mg/kg, i.p.) (Figure 4). In spinal cord synaptosomal preparations, the results showed that the treatment with ULI increased AMP hydrolysis only; ATP and ADP hydrolysis remained unaltered (Figure 3(a), ATP: F(2,17) = 5.579, p < 0.05; ADP: F(2,17) = 3.327, p = 0.071; AMP: F(2,17) = 6.921, p < 0.01). In cerebral cortex synaptosomes, the treatment with ULI increased the ATP hydrolysis and there were no changes on ADP and AMP hydrolysis (Figure 3(b), ATP: F(2,14) = 5.579, p < 0.05; ADP: F(2,14) = 3.327, p = 0.071; AMP: F(2,14) = 0.934, p = 0.420).

4. Discussion

Herein we demonstrated for the first time the involvement of the purinergic system in the antinociceptive effect of uliginosin B (ULI), a dimeric acylphloroglucinol from Hypericum species native to South America. Previous data showed that ULI (15 mg/kg, i.p.) produces antinociceptive effect in the hot-plate test [3, 5, 6]. We now show that the pretreatment with DPCPX and ZM 241385, selective adenosine A₁ and A₂A receptor antagonists, respectively, completely prevented the antinociceptive effect of ULI in the mice hot-plate test. This finding indicates that the activation of these receptors mediates the effect of ULI. However, ULI does not have the classical structural requirements for binding to adenosine receptors, since it lacks nitrogen atoms and amine groups, which seem to be crucial for ligands of

![Figure 1: Uliginosin B structure (a). Hypericum polyanthemum, plant used to obtain ULI (b).](image-url)
these receptors [37–40]. Another possibility could be due to allosteric interactions. Nevertheless, although the structure activity relationship is still not completely established, the main allosteric modulators of adenosine receptors also contain nitrogen atoms or amine groups. In addition, compounds that act allosterically and/or orthostERICALLY at the A₁ adenosine receptor have often close structural resemblance, which suggests that the allosteric site on the A₁ adenosine receptor is closer or very similar to the orthosteric site of this receptor [41]. Thus, we supposed that the activation of adenosine receptors following ULI treatment could result from increased adenosine availability.

As already mentioned, previous studies by some of us demonstrated that ULI has antidepressant-like effect by inhibiting synaptosomal monoamines reuptake and possibly enhancing the extracellular monoamine availability [2]. Amitriptyline and desipramine, which are antidepressants that increase the availability of monoamines, displayed
antinociceptive effects dependent of adenosine receptors activation [8–10, 42–44]. In addition, antidepressants that increase the extracellular availability of monoamines seem to modulate nucleotide hydrolysis in the central nervous system (CNS), presenting stimulatory or inhibitory effect depending on the treatment duration and brain structure [45–47]. The effects of antidepressants on adenosine system seem to reflect increased availability of adenosine following an effect on transport and not necessarily effects on amine transporters [48]. Therefore, we decided to investigate the effect of ULI on nucleotide hydrolysis and adenosine metabolism in order to observe whether adenosine availability has influence on the antinociceptive properties of this compound.

It is noteworthy that our data indicate that the antinociceptive effect of ULI could be, at least in part, dependent of adenosine availability, since it was prevented by pretreatment with AMP CP, an ecto-5'-nucleotidase inhibitor. This result was confirmed by the ex vivo assay in spinal cord synaptosomes which pointed to an increase in AMP hydrolysis induced by ULI. In cerebral cortex synaptosomes, treatment with ULI increased the ATP hydrolysis and there were no changes on ADP and AMP hydrolysis. These different effects on AMP hydrolysis could be due to an increased expression of ecto-5'-nucleotidase in the cerebral cortex [49] or to a different processing of the protein, which has been shown to be present in different isoforms in nerve terminals [50, 51], or instead to an abrogation of the negative allosteric modulation of this enzymatic activity by adenine nucleotides [52]. Spinal cord and cerebral cortex, which have been considered as important antinociceptive pathways, possess a high density of adenosine receptors [11, 53, 54].

The fact that ULI stimulates ATP hydrolysis without altering ADP hydrolysis in the cerebral cortex agrees with other studies. ATP, through activation of P_{2X1} receptors, generally facilitates nociceptive transmission while ADP (via P_{2Y} receptors) may decrease the excitatory effect of ATP [55, 56]. ATP can facilitate nociceptive sensitivity by the activation of both ATP-gated ion channels (P2X receptors) and G protein-coupled (P2Y) receptors contributing to nociceptive signaling in peripheral sensory neurons. On the other hand, Gi-coupled P2Y receptors activation can modulate pain neurotransmission [57].

An extensive review by Cunha [58] has pointed that the activation of adenosine A1 and A2A receptors is proven to be associated with the release of monoamines, glutamate, and other neuromodulators in different brain regions. In addition, the release of adenosine may be modulated by activation of these neurotransmitter pathways [11, 59–62], as well as opioids [63–66]. Quarta and coworkers [67] have postulated a circuit under physiological conditions of high adenosine release, where regulation of the activation of adenosine A1 and A2A receptors could induce glutamate and dopamine release. In this context, it is possible to hypothesize that an increase in adenosine levels could be responsible for an increase in the availability of monoamines and activation of glutamate and opioid receptors, previously described for ULI [2, 3, 5, 6]. Further studies are needed in order to substantiate this assumption.

As a final point, our results demonstrate that the ULI effects on adenosine metabolism involve mainly the modulation of adenosine levels by ecto-5'-nucleotidase activity, since the nociceptive response of this phloroglucinol derivative was not altered by pretreatment with dipyridamole and EHNA, which are nucleoside transporter and ADA inhibitors, respectively. In addition, considering that ATP plays a key role as a danger signal in the brain [68], the ULI ability to increase ATP hydrolysis, with consequent generation of adenosine, may indicate a neuroprotective effect. On the other hand, the
results so far do not rule out the possibility that ULI could be a trigger of ATP release. Further experiments are planned in order to investigate these outcomes.

5. Conclusion

In conclusion, the present results indicate that uliginosin B increases the availability of adenosine, via ecto-5’-nucleotidases, with consequent activation of adenosine receptors (particularly $A_1$ and $A_{2A}$), which play a role in the antinociceptive effect of this phloroglucinol. These findings opened a new avenue for searching the mode of action of this original neuroactive molecular pattern.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

The authors are grateful to Sergio Bordignon, Ph.D., for collecting and identifying the plant material and to CAPES/Brazil and CNPq/Brazil for financial support.

References

[1] G. V. Ccana-Ccapatinta, F. M. C. de Barros, H. Bridi, and G. L. von Posser, “Dimeric acylphloroglucinols in Hypericum species from sections Brathys and Trignobrathys,” Phytochemistry, vol. 14, no. 1, pp. 23–50, 2015.

[2] A. C. Stein, A. F. Viana, L. G. Müller et al., “Uliginosin B, a phloroglucinol derivative from Hypericum polyanthenum: a promising new molecular pattern for the development of antidepressant drugs,” Behavioural Brain Research, vol. 228, no. 1, pp. 66–73, 2012.

[3] E. D. Stolz, A. F. Viana, D. R. Hasse, G. L. von Posser, J.-C. do Rego, and S. M. K. Rates, “Uliginosin B presents antinociceptive effect mediated by dopaminergic and opioid systems in mice,” Progress in Neuro-Psychopharmacology and Biological Psychiatry, vol. 39, no. 1, pp. 80–87, 2012.

[4] C. Socolsky, S. M. K. Rates, A. C. Stein, Y. Asakawa, and A. Bardón, “Acylphloroglucinols from Elaphoglossum crassipes: antidepressant-like activity of crassispin A,” Journal of Natural Products, vol. 75, no. 6, pp. 1007–1017, 2012.

[5] E. D. I. Stolz, D. R. A. Hasse, G. L. I. von Posser, and S. M. K. Rates, “Uliginosin B, a natural phloroglucinol derivative, presents a multimodal antinociceptive effect in mice,” The Journal of Pharmacy and Pharmacology, vol. 66, no. 12, pp. 1774–1785, 2014.

[6] E. D. Stolz, L. G. Müller, C. B. Antonio et al., “Determination of pharmacological interactions of uliginosin B, a natural phloroglucinol derivative, with amitriptyline, clonidine and morphine by isobolographic analysis,” Phytomedicine, vol. 21, no. 12, pp. 1684–1688, 2014.

[7] J. Sawynok, “Adenosine receptor targets for pain,” Neuroscience, 2015.

[8] M. J. Esser, T. Chase, G. V. Allen, and J. Sawynok, “Chronic administration of amitriptyline and caffeine in a rat model of neuropathic pain: multiple interactions,” European Journal of Pharmacology, vol. 430, no. 2-3, pp. 211–218, 2001.

[9] J. Sawynok, A. R. Reid, and M. J. Esser, “Peripheral antinociceptive action of amitriptyline in the rat formalin test: involvement of adenosine,” Pain, vol. 80, no. 1-2, pp. 45–55, 1997.

[10] A. Ulugol, H. C. Karadag, M. Tamer, Z. Fırat, A. Aslantas, and I. Dokmeci, “Involvement of adenosine in the anti-allodynic effect of amitriptyline in streptozotocin-induced diabetic rats,” Neuroscience Letters, vol. 328, no. 2, pp. 129–132, 2002.

[11] J. Sawynok and X. J. Liu, “Adenosine in the spinal cord and periphery: release and regulation of pain,” Progress in Neurobiology, vol. 69, no. 5, pp. 313–340, 2003.

[12] G. J. Keil and G. E. Delander, “Time-dependent antinociceptive interactions between opioids and nucleoside transport inhibitors,” Journal of Pharmacology and Experimental Therapeutics, vol. 274, no. 3, pp. 1387–1392, 2014.

[13] T. W. Stone, “The effects of morphine and methionine-enkephalin on the release of purines from cerebral cortex slices of rats and mice,” British Journal of Pharmacology, vol. 74, no. 1, pp. 171–176, 1981.

[14] K. O. Aley and J. D. Levine, “Multiple receptors involved in peripheral $\alpha_2$, $\mu$, and $\delta$ antinociception, tolerance, and withdrawal,” The Journal of Neuroscience, vol. 17, no. 2, pp. 735–744, 1997.

[15] G. Burnstock and M. Williams, “P2 purinergic receptors: modulation of cell function and therapeutic potential,” Journal of Pharmacology and Experimental Therapeutics, vol. 295, no. 3, pp. 862–869, 2000.

[16] G. Burnstock, “Physiology and pathophysiology of purinergic neurotransmission,” Physiological Reviews, vol. 87, no. 2, pp. 659–797, 2007.

[17] S. C. Robson, J. Sévigny, and H. Zimmermann, “The E-NTPDase family of ectonucleotidases: structure function relationships and pathophysiological significance,” Purinergic Signalling, vol. 2, no. 2, pp. 409–430, 2006.

[18] M. J. Zylka, “Pain-relieving prospects for adenosine receptors and ectonucleotidases,” Trends in Molecular Medicine, vol. 17, no. 4, pp. 188–196, 2011.

[19] G. Burnstock, “Introduction to purinergic signalling in the brain,” Advances in Experimental Medicine and Biology, vol. 986, pp. 1–12, 2013.

[20] P. L. Ipata, “Origin, utilization, and recycling of nucleosides in tissue culture-derived Hypericum uliginosum. I,” Journal of Pharmacy and Pharmacology, vol. 66, no. 12, pp. 1774–1785, 2014.

[21] C. D. Bonan, “Ectonucleotidases and nucleotide/nucleoside transporters as pharmacological targets for neurological disorders,” CNS and Neurological Disorders—Drug Targets, vol. 11, no. 6, pp. 739–750, 2012.

[22] J. D. M. Nunes, A. V. Pinhatti, G. L. von Posser, and S. B. Rech, “Promotive effects of long-term fertilization on growth of tissue culture-derived Hypericum polyanthenum plants during acclimatization,” Industrial Crops and Products, vol. 30, no. 2, pp. 329–332, 2009.

[23] W. L. Parker and F. Johnson, “The structure determination of antibiotic compounds from Hypericum uliginosum. I,” Journal of the American Chemical Society, vol. 90, no. 17, pp. 4716–4723, 1968.

[24] Ministério da Ciência e Tecnologia e Inovação (Brasil), “Lei procedimentos para o uso científico de animais,” Lei n. 11794, Diário Oficial da União (DOU), 2008.

[25] Brasil: Ministério da Ciência Tecnologia e Inovação—CONCEA, Diretriz Brasileira para o Cuidado e a Utilização de Animais para Fins Científicos e Didáticos—DBCA, Ministério
Evidence-Based Complementary and Alternative Medicine

da Ciência Tecnologia e Inovação, Brasilia, Brazil, 2013, http://www.mct.gov.br/upd_bloob/0226/226494.pdf.

[26] Brasil, Ministério da Ciência Tecnologia e Inovação, CONCEA. Diretrizes da prática de eutanásia do CONCEA. Brasília-DF, 2013, http://www.mct.gov.br/upd_bloob/0226/226746.pdf.

[27] Committee for the Update of the Guide for the Care and Use of Laboratory Animals Institute for Laboratory Animal Research Division on Earth and Life Studies, Laboratory Guide for the Care and Use of Animals, The National Academies Press, 2011.

[28] F. P. Nascimento, S. M. Figueredo, R. Marcon et al., “Inosine reduces pain-related behavior in mice: involvement of adenine A1 and A2A receptor subtypes and protein kinase C pathways,” Journal of Pharmacology and Experimental Therapeutics, vol. 334, no. 2, pp. 590–598, 2010.

[29] D. F. Martins, L. Mazzardo-Martins, F. Soldi, J. Stramosk, A. P. Piovezan, and A. R. S. Santos, “High-intensity swimming exercise reduces neuropathic pain in an animal model of complex regional pain syndrome type I: evidence for a role of the adenosinergic system,” Neuroscience, vol. 234, pp. 69–76, 2013.

[30] H. Hosseinizadeh and M. E. Shooshtari, “Antinociceptive interaction between adenosine and carbamazepine in mice,” Medical Journal of the Islamic Republic of Iran, vol. 12, no. 4, pp. 381–386, 1999.

[31] A. Grenz, H. Zhang, J. Weingart et al., “Lack of effect of extracellular adenosine generation and signaling on renal erythropoietin secretion during hypoxia,” American Journal of Physiology—Renal Physiology, vol. 293, no. 5, pp. 1501–1511, 2007.

[32] J. R. Rozisky, R. S. da Silva, L. S. Adachi et al., “Neonatal morphine exposure alters E-NTPDase activity and gene expression pattern in spinal cord and cerebral cortex of rats,” European Journal of Pharmacology, vol. 642, no. 1–3, pp. 72–76, 2010.

[33] A. Nagy and A. V. Delgado-Escueta, “Rapid preparation of synaptosomes from mammalian brain using nontoxic isosmotic gradient material (Percoll),” Journal of Neurochemistry, vol. 43, no. 4, pp. 1114–1123, 1984.

[34] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," Analytical Biochemistry, vol. 72, no. 1-2, pp. 248–254, 1976.

[35] A. M. O. Battastini, J. B. T. da Rocha, C. K. Barcellos, R. D. Dias, and J. J. F. Sarkis, “Characterization of an ATP diphosphohydrolase (EC 3.6.1.5) in synaptosomes from cerebral cortex of adult rats,” Neurochemical Research, vol. 16, no. 12, pp. 1303–1310, 1991.

[36] K.-M. Chan, D. Delfert, and K. D. Junger, "A direct colorimetric assay for Ca"-stimulated ATPase activity," Analytical Biochemistry, vol. 157, no. 2, pp. 375–380, 1986.

[37] A. P. Ijzerman, P. J. M. Van Galen, and K. A. Jacobson, “Molecular modeling of adenosine receptors. I. The ligand binding site on the A1 receptor,” Drug Design and Discovery, vol. 9, no. 1, pp. 49–67, 1992.

[38] J. Kim, J. Wess, A. M. Van Rhee, T. Schöneberg, and K. A. Jacobson, "Site-directed mutagenesis identifies residues involved in ligand recognition in the human A2a adenosine receptor," Journal of Biological Chemistry, vol. 270, no. 23, pp. 13987–13997, 1995.

[39] A. J. Kooistra, L. Roumen, R. Leurs, I. J. P. de Esch, and C. de Graaf, "From heptahedral bundle to hits from the haystack: structure-based virtual screening for GPCR ligands," Methods in Enzymology, vol. 522, pp. 279–336, 2013.

[40] K. A. Jacobson, "Structure-based approaches to ligands for G-protein-coupled adenosine and P2Y receptors, from small molecules to nanoconjugates," Journal of Medicinal Chemistry, vol. 56, no. 10, pp. 3749–3767, 2013.

[41] A. Góblóyos and A. P. Ijzerman, “Allosteric modulation of adenosine receptors,” Biochimica et Biophysica Acta (BBA)—Biomembranes, vol. 1808, no. 5, pp. 1309–1318, 2011.

[42] J. Sawynok and A. Reid, “Desipramine potentiates spinal antinociception by 5-hydroxytryptamine, morphine and adenosine,” Pain, vol. 50, no. 1, pp. 113–118, 1992.

[43] J. Sawynok, A. R. Reid, and B. B. Fredholm, “Caffeine reverses antinociception by amitriptyline in wild type mice but not in those lacking adenosine A1 receptors,” Neuroscience Letters, vol. 440, no. 2, pp. 181–184, 2008.

[44] M. J. Esser and J. Sawynok, “Caffeine blockade of the thermal antihyperalgesic effect of acute amitriptyline in a rat model of neuropathic pain,” European Journal of Pharmacology, vol. 399, no. 2-3, pp. 131–139, 2000.

[45] E. L. Pedrazza, M. R. Senger, E. P. Rico et al., “Fluoxetine and nortriptyline affect NTPDase and 5’-nucleotidase activities in rat blood serum,” Life Sciences, vol. 81, no. 15, pp. 1205–1210, 2007.

[46] E. L. Pedrazza, M. R. Senger, L. Pedrazza, F. F. Zimmermann, J. J. de Freitas Sarkis, and C. D. Bonan, “Sertraline and clomipramine inhibit nucleotide catabolism in rat brain synaptosomes,” Toxicology in Vitro, vol. 21, no. 4, pp. 671–676, 2007.

[47] E. L. Pedrazza, E. P. Rico, M. R. Senger et al., “Ecto-nucleotidase pathway is altered by different treatments with fluoxetine and nortriptyline,” European Journal of Pharmacology, vol. 583, no. 1, pp. 18–25, 2008.

[48] J. Sawynok, A. R. Reid, J. L. Xue, and F. E. Parkinson, “Amitriptyline enhances extracellular tissue levels of adenosine in the rat hindpaw and inhibits adenosine uptake,” European Journal of Pharmacology, vol. 518, no. 2-3, pp. 116–122, 2005.

[49] K. Kohring and H. Zimmermann, "Upregulation of ecto-5'-nucleotidase in human neuroblastoma SH-SY5Y cells on differentiation by retinoic acid or phorbol ester," Neuroscience Letters, vol. 258, no. 3, pp. 127–130, 1998.

[50] R. Napieralski, B. Kempske, and W. Gutensohn, “Evidence for coordinated induction and repression of ecto-5'-nucleotidase (CD73) and the A2a adenosine receptor in a human B cell line,” Biological Chemistry, vol. 384, no. 3, pp. 483–487, 2003.

[51] R. A. Cunha, P. Brendel, H. Zimmermann, and J. A. Ribeiro, “Immunologically distinct inosine- and adenosine-5'-nucleotidase in nerve terminals of different areas of the rat hippocampus,” Journal of Neurochemistry, vol. 74, no. 1, pp. 334–338, 2000.

[52] R. A. Cunha, “Regulation of the ecto-nucleotidase pathway in rat hippocampal nerve terminals,” Neurochemical Research, vol. 26, no. 8-9, pp. 979–991, 2001.

[53] J. D. Geiger, F. S. LaBella, and J. I. Nagy, “Characterization and localization of adenosine receptors in rat spinal cord,” The Journal of Neuroscience, vol. 4, no. 9, pp. 2303–2310, 1984.

[54] N. A. Sowa, B. Taylor-Blake, and M. J. Zylka, “Ecto-5’-nucleotidase (CD73) inhibits nociception by hydrolyzing AMP to adenosine in nociceptive circuits,” The Journal of Neuroscience, vol. 30, no. 6, pp. 2235–2244, 2010.

[55] H. Z. Ruan and G. Burnstock, “Localization of P2Y1 and P2Y4 receptors in dorsal root, nodose and trigeminal ganglia of the rat,” Histochemistry and Cell Biology, vol. 120, no. 5, pp. 415–426, 2003.
[56] Z. Gerevich and P. Illes, “P2Y receptors and pain transmission,” Purinergic Signalling, vol. 1, no. 1, pp. 3–10, 2004.

[57] S. A. Malin and D. C. Molliver, “Gi- and Gq-coupled ADP (P2Y) receptors act in opposition to modulate nociceptive signaling and inflammatory pain behavior,” Molecular Pain, vol. 6, no. 21, pp. 1–12, 2010.

[58] R. A. Cunha, “Adenosine as a neurmodulator and as a homeostatic regulator in the nervous system: different roles, different sources and different receptors,” Neurochemistry International, vol. 38, no. 2, pp. 107–125, 2001.

[59] M. Sweeney, T. White, and J. Sawynok, “5-Hydroxytryptamine releases adenosine from primary afferent nerve terminals in the spinal cord,” Brain Research, vol. 462, no. 2, pp. 346–349, 1988.

[60] M. I. Sweeney, T. D. White, and J. Sawynok, “5-Hydroxytryptamine releases adenosine and cyclic AMP from primary afferent nerve terminals in the spinal cord in vivo,” Brain Research, vol. 528, no. 1, pp. 55–61, 1990.

[61] M. A. da Silva Pereira, Estudo da Atividade Antinociceptiva de Adenosina em Camundongos—Análise do Mecanismo de Ação, Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade do Vale do Itajaí, Florianópolis, Brazil, 2005.

[62] M. J. Millan, “Descending control of pain,” Progress in Neurobiology, vol. 66, no. 6, pp. 355–474, 2002.

[63] J. Sawynok, M. I. Sweeney, and T. D. White, “Adenosine release may mediate spinal analgesia by morphine,” Trends in Pharmacological Sciences, vol. 10, no. 5, pp. 186–189, 1989.

[64] J. Sawynok, D. J. Nicholson, M. I. Sweeney, and T. D. White, “Adenosine release by morphine and spinal antinociception: role of G-proteins and cyclic AMP,” NIDA Research Monograph Series, vol. 105, pp. 40–46, 1990.

[65] J. Sawynok, “Adenosine receptor activation and nociception,” European Journal of Pharmacology, vol. 347, no. 1, pp. 1–11, 1998.

[66] J. Sawynok, A. Reid, and A. Poon, “Peripheral antinociceptive effect of an adenosine kinase inhibitor, with augmentation by an adenosine deaminase inhibitor, in the rat formalin test,” Pain, vol. 74, no. 1, pp. 75–81, 1998.

[67] D. Quarta, J. Borycz, M. Solinas et al., “Adenosine receptor-mediated modulation of dopamine release in the nucleus accumbens depends on glutamate neurotransmission and N-methyl-D-aspartate receptor stimulation,” Journal of Neurochemistry, vol. 91, no. 4, pp. 873–880, 2004.

[68] R. J. Rodrigues, A. R. Tomé, and R. A. Cunha, “ATP as a multitarget danger signal in the brain,” Frontiers in Neuroscience, vol. 9, article 148, 2015.