Débora dos Santos Silva

A dupla face dos corticoides sobre o sistema melatonérgico de células imunocompetentes

The double face of corticoids upon the melatonergic system of immunocompetent cells

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Orientador: Prof. Dr. Pedro Augusto Carlos Magno Fernandes

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Lista de Abreviaturas e siglas

5-HTP	**5-hidroxitriptofano**
5-HT	**serotonina**
AANAT	**arilalquilamina N-acetiltransferase**
ACTH	**hormônio adrenocorticotrófico**
AMP	**adenosina monofosfato**
AMPc	**monofosfato cíclico de adenosina**
ASMT	**N-acetylserotonina-O-metiltransferase**
ATP	**adenosina trifosfato**
CBP	**CREB binding protein**
CORT	**corticosterona**
CRE	**elementos responsivos a AMPc**
CREB	**cyclic AMP response element binding**
CRH	**hormônio corticotrófico**
DAMPs	**padrões moleculares associados a perigo**
DNA	**ácido desoxirribonucleico**
EIP	**eixo imune pineal**
epm	**erro padrão da média**
GPCRs	**receptores acoplados à proteína G**
GR	**receptores para glicocorticoides**
GRE	**regiões responsivas a glicocorticoides**

Gs	**proteína G estimulatória**
HHA	**eixo hipotálamo – hipófise – adrenal**
HIOMT	**hidroxi-indol-O-metiltransferase**
HPA	**eixo hipotálamo – pituitária – adrenal**
hsp	**heat shock proteins**
IgG	**imunoglobulina G**
IkB	**proteína inibitória κB**
IFN-α	**interferon α**
IFN-γ	**interferon γ**
IL-1	**Interleucina 1**
IL-2	**Interleucina 2**
IL-4	**Interleucina 4**
IL-6	**Interleucina 6**
IL-10	**Interleucina 10**
LBD	**ligand binding domain**
LPS	**lipopolissacarideo de bactérias Gram-negativas**
MEL	**melatonina**
mL	**mililitro**
MR	**receptores de mineralocorticoides**
MT1	**receptor de melatonina do subtipo 1**
MT2	**receptor de melatonina do subtipo 2**
NAd	**noradrenalina**

NAS	**N-acetilserotonina**
NFκB	**fator nuclear Kappa B**
NK	**natural killer**
nM	**nanomolar**
PAMPs	**padrões moleculares associados a patógenos**
PBS	**salina tamponada com fosfato**
PKA	**proteína quinase dependente de AMP cíclico**
PMA	***acetato miristato* de *forbol***
PVN	**núcleo paraventricular do hipotálamo**
RNA	**ácido ribonucleico**
RU 486	**mifepristona**
Spiro/Sp	**Spirolactona**
STAT	**signal tranducer and activator of transcription**
TAD	**domínio de ativação de transcrição**
TNF	**fator de necrose tumoral**
TPH1	**triptofano hidroxilase 1**
TLR	**receptor do tipo Toll 2**
TLR	**receptor do tipo Toll 4**
TLR	**receptor do tipo Toll 6**
zy	**zimosan**
µg	**micrograma**
µM	**micromolar**

RESUMO

A melatonina (MEL) é um hormônio produzido e secretado pela glândula pineal. Além da produção noturna por essa glândula, as células do sistema imunológico também produzem MEL. Vários estudos demonstram que a produção de MEL pela pineal e células imunes são controladas por agentes endógenos (TNF, catecolaminas) e exógenos (LPS, zymosan) que ativam/controlam o desenvolvimento de processos de defesa. Em macrófagos, a produção de MEL induzida por zimosan, por exemplo, aumenta a fagocitose dessas células. Na glândula pineal, os glicocorticoides podem aumentar ou reduzir a síntese de MEL, dependendo do padrão de estimulação adrenérgica imposta à glândula. Em macrófagos RAW 264.7, observamos um duplo efeito da corticosterona (CORT) dependendo do padrão de estimulação, que pode potencializar ou inibir a produção de melatonina. Nossos resultados com células peritoneais e de medula óssea também mostraram um efeito contexto-específico, onde observamos diferentes efeitos na expressão da enzima dependendo das concentrações de CORT e dexametasona (DEXA) e do tipo de estímulo que está atuando nas células. Nossos dados mostram o sinergismo entre receptores de glicocorticoides (GR) e de mineralocorticoides (MR) tanto na ativação do sistema melatonérgico quanto na inibição do sistema quando ativado pela via do NFκB. Esses achados ampliam nossos entendimentos da relação CORT/MEL e podem servir de base para estudos e terapias futuras.

Palavras-chave: Melatonina; Corticosterona; Dexametasona; Zimosan; receptores de glicocorticoides e de mineralocorticoides.

ABSTRACT

Melatonin (MEL) is a hormone produced and secreted by the pineal gland. In addition to nocturnal production by this gland, immune system cells also produce MEL. Several studies demonstrate that MEL production by the pineal and immune cells are controlled by endogenous (TNF, catecholamines) and exogenous (LPS, zymosan) agents that activate/control the development of defense processes. In macrophages, zymosan -induced MEL production, for example, enhances the phagocytosis of these cells. In the pineal gland, glucocorticoids can either enhance or reduce MEL synthesis depending on the pattern of adrenergic stimulation imposed on the gland. In RAW 264.7 macrophages, we observed a dual effect of corticosterone (CORT) depending on the stimulatory pattern, which can either potentiate or inhibit melatonin production. Our results with peritoneal and bone marrow cells also showed a context-specific effect, where we observed different effects on enzyme expression depending on the concentrations of CORT and dexamethasone (DEXA) and the type of stimulus that is acting on the cells. Our data show the synergism between glucocorticoid (GR) and mineralocorticoid (MR) receptors both in activating the melatonergic system and inhibiting the system when activated by the NFκB pathway. These findings broaden our understanding of the CORT/MEL relationship and may serve as a basis for future studies and therapies.

Keywords: Melatonin; Corticosterone; Dexamethasone; Zimosan; glucocorticoid/mineralocorticoid receptors.

1.GENERAL INTRODUCTION

1.1. Pineal and extra-pineal production of melatonin

Melatonin (MEL) is a hormone produced and secreted by the pineal gland. The circadian production of this hormone is essential for the adaptation of individuals to the cyclical temporal variations of the environment, being associated, for example, with the regulation of the sleep-wake and reproductive cycles (Soares *et al*.,2003; Morris *et al*., 2012; Fisher *et al*., 2013; Maitra & Hasan, 2016) and to control the functioning of the cardiovascular and immune systems (Macchi *et al*., 2004; Markus *et al*., 2007).

The synthesis of MEL by the pineal occurs in the dark phase and is dependent on the activation of the sympathetic autonomic nervous system and the consequent release of noradrenaline (NAd) by the terminals that innervate this gland (Fálcon, 1999). In the pineal, NAd interacts with postsynaptic b1 and α1-adrenergic receptors in pinealocytes, triggering a series of intracellular biochemical events that lead to the synthesis of melatonin (Klein, 1985). In this process, tryptophan captured from the circulation is converted into 5-hydroxytryptophan (5-HTP) under the action of the enzyme tryptophan hydroxylase 1 (TPH1) (Lovenberg et al., 1967). 5-HTP will be decarboxylated by the enzyme 5-HTP decarboxylase, giving rise to serotonin (5-HT) (Snyder & Axelrod, 1964). Serotonin, in turn, is acetylated by the action of the enzyme aralkylamine-N-acetyltransferase (AANAT), giving rise to N-acetylserotonin (NAS) (Voisin et al., 1984), which the enzyme hydroxyindole-O-methyltransferase (HIOMT, also called N-acetylserotonin-O-

methyltransferase, ASMT), will later methylate, forming melatonin (Ribelayga & Simonneaux, 2003).

There are also extra pineal sources of MEL described in the literature, in addition to the pineal gland, such as the retina (Cardinali & Rosner, 1971), gastrointestinal tract (Raikhlin et al., 1975), organs and cells of the immune system, such as the thymus, spleen, lymphocytes, mast cells, leukocytes, platelets and endothelial cells (Kvetnoy, 1999). Interestingly, MEL production was observed in the central nervous system (Pinato et al., 2015), in astrocytes from rats of the C6 glioma lineage (Liu et al., 2007), and in human cell lines of brain tumors (Kinker et al., 2016, 2021). In these cases, the local melatonin production protected the brain from inflammation-derived damage (Pinato et al., 2015) and decreased tumor cell proliferation (Kinker et al., 2016, 2021), pointing out the regulatory and protective roles of the melatonergic system.

The current understanding is that the melatonergic system adjusts body physiology to recurrent environmental changes and unpredictable stressful variations, such as trauma and exposure to pathogens. Therefore, several studies point out that melatonin is produced by immune cells and modulates several immune functions. For example, MEL exerts protective effects on the thymus of rats by modulating apoptosis processes and cell proliferation (Gomez-Corvera et al., 2009; Sanchez-Hidalgo et al., 2009; Sokolovic et al., 2013) and protects mast cells against cytotoxicity (Maldonado et al., 2013). In human lymphocytes, cellular production of MEL plays a crucial role in modulating the IL2/IL2R receptor system and the IL2-mediated lymphocyte maturation (Carrilo-Vico et al., 2004). Martins and col (2004) observed the production of serotonin and MEL in the presence of tryptophan

in peritoneal macrophages. The cells exhibited high expression of AANAT and MEL, a process triggered by interferon α and γ (IFN-α / IFN-γ), phorbol myristate acetate (PMA), and lipopolysaccharide (LPS). In addition to peritoneal macrophages, the production of MEL by other types of macrophages has already been demonstrated, such as human colostrum macrophages (Pontes et al., 2006; Pires-Lapa et al., 2013) and in macrophages of the lineage RAW264.7 (Muxel et al., 2012).

In the early 2000s, Tan and collaborators observed high melatonin production in rats' bone marrow and described the presence of the enzymes AANAT and HIOMT. Similar results were described by Conti et al., 2000 with human bone marrow cells and in different cell lines from mice. High melatonin concentrations in long-term (four weeks) bone marrow cultures also suggested endogenous melatonin synthesis. In a recent work published by our research group, we demonstrated a circadian variation in the expression of melatonin biosynthetic enzymes and the production of melatonin in the bone marrow (Córdoba-Moreno et al., 2020). Interestingly, MEL produced by the pineal gland acts in endothelial cells reducing the rolling and adhesion of neutrophils (Tamura et al., 2010), suggesting that the local melatonin production by immunocompetent cells might also be adjusting the migration of cells from different compartments. Accordingly, rhythmically-produced melatonin by immune cells in the bone marrow (Cordoba-Moreno et al., 2020) controls the differentiation of stem cells and promotes cellular self-renewing by inhibiting cellular migration to the blood (Golan et al., 2018).

The modulation and effects of melatonin produced by the pineal and immunocompetent cells in healthy situations and responses to immunological

stimuli formed the experimental framework that allowed the description of the Immune-Pineal Axis (IPA, Markus et al., 2007, 2018).

1.2. The Immune-Pineal Axis

In the '70s, the first indications that the pineal gland is related to the modulation of the immune system were noticed (Klein et al., 1972). These studies demonstrated that pinealectomized animals accelerated the involution of the thymus, the organ of the immune system responsible for the maturation of lymphocytes (Klein et al., 1972; Klein, 1979). Studies have shown that melatonin acts as a crucial immunomodulatory agent, increasing T lymphocyte proliferation, antigen presentation, and macrophage phagocytosis (Carrillo-Vico et al., 2005; Pontes et al., 2006; Pires-Lapa et al., 2006; Pires-Lapa et al., 2013; Markus & Cecon, 2013; Carrilo-Vico et al., 2013).

Given the interdependence between the melatonin-producing systems and the immune system, our working group has evaluated the role of melatonin as an integral part of regulating immune processes. As mentioned earlier, the dataset obtained so far allowed the perception and description of the IPA (Markus et al., 2007, 2018). In this context, melatonin produced by the pineal gland partially inhibits the migration of leukocytes from the bloodstream to the tissues (Lotufo et al., 2001; Marçola et al., 2012) and, during inflammatory processes, exogenous agents (e.g., LPS from gram bacteria, Da Silveira Cruz Machado et al., 2010) and endogenous signals (e.g., TNF, Fernandes, et al., 2006) transiently reduce the pineal`s production of MEL by a mechanism dependent on the activation of the nuclear factor κB (NFκB). The decrease in circulating MEL levels results in stronger adhesion

and migration of immunocompetent cells through the vascular endothelium (Tamura et al., 2010; Marçola et al., 2012) is an essential regulatory mechanism for the assembly and migration of cells, promoting inflammatory process mounting.

At the site of inflammation, immunocompetent cells such as lymphocytes (Carrillo-Vico et al., 2005) and macrophages (Muxel et al., 2012 and Pires-Lapa et al., 2013) produce high concentrations of melatonin in response to pathogen-associated molecular patterns (PAMPs) such as LPS (Muxel et al., 2012) and the β-glucan of the fungal cell wall, zymosan (Pires-Lapa et al., 2013). Accordingly, studies carried out in human colostrum immunocompetent cells show the melatonin production induced by enteropathogenic Escherichia coli bacteria (Pontes et al., 2006) and zymosan (Pontes et al., 2006, 2007). LPS and zymosan activate the translocation of NFκB in colostrum and lineage macrophages (RAW 264.7), and the use of an inhibitor of this pathway inhibits the production of melatonin induced by these PAMPs (Muxel et al., 2012 and Pires-Lapa et al., 2013). Furthermore, zymosan induces in RAW macrophages an increase in NFκB cRel, RelA, and p50 subunits in the form of p50/RelA and p50/cRel dimmers (Muxel et al., 2012). Thus, it is interesting to note that NFκB is fundamental in several aspects of the IPA activation during the inflammatory process, as it inhibits the production of melatonin by the pineal (allowing more significant migration of cells to the focus of the lesion) and stimulates local production by immunocompetent cells (Markus et al., 2013).

As mentioned before, melatonin production by immunocompetent cells regulates several aspects of the defense system. Besides the role of

melatonin in the IL2/IL2R receptor system during lymphocyte maturation (Carrilo-Vico et al., 2004) and the control of stem cell differentiation and cellular self-renewing in the bone marrow (Golan et al., 2018), another exciting effect exert by local production of melatonin is the regulation phagocytosis. In colostrum phagocytic mononuclear cells, for example, exogenous and endogenous melatonin has been shown to positively control the phagocytic capacity of these cells by a melatonin receptor-dependent mechanism (Pires-Lapa et al., 2013). Moreover, melatonin enhances the phagocytosis of diesel particles by alveolar macrophages (Carvalho-Souza et al., 2020) and the PAMP-induced phagocytic activity of microglial cells (Souza et al., 2022).

Therefore, a relevant frontier of research of the IPA focuses on understanding the signals and mechanisms that regulate melatonin production by different types of immunocompetent cells. Besides the aforementioned stimulatory roles of the transactivation domain positive (TAD+) NFκB (cRel, RelA) subunits, there also evidences that the STAT pathway, activated by IFNg and IL10, also regulates the production of melatonin by immune cells (Martins et al., 2004, Cordoba-Moreno et al., 2020). Interestingly, the activation of STATs adjusts pineal function by interacting with the NFκB pathway (Barbosa-Lima et al., 2019, Cordoba-Moreno et al., in preparation). Another relevant transcription factor that adjusts melatonin production and interacts with the NFκB signaling is the glucocorticoid receptor (GR) triggered by glucocorticoids. In the next section, we will explore this pathway's role in controlling melatonin production, which formed the present thesis's groundwork.

1.3. Glucocorticoid effects upon melatonin synthesis

During unbalanced infection processes, the excessive production of circulating cytokines can cause the host's death (Hack et al., 1997), pointing to the importance of appropriate regulation of the immune response in time and intensity. The counterpoint of the pro-inflammatory response is the action of regulatory modulators, such as glucocorticoids (Turnbull & Rivier, 1999). Thus, the dynamics of a defense process have, in its functional basis, the activation of the hypothalamic-pituitary/pituitary-adrenal (HHA/HPA) axis and the consequent release of glucocorticoids. The effects of glucocorticoids were first observed in 1855 by the British physician Thomas Addison, who observed that lesions in the adrenal glands led to the death of patients, thus describing a syndrome resulting from the destruction of the adrenals, which later took his name, syndrome of Addison (Smans & Zelissen, 2012). The following year, the physiologist Charles Édouard Brown-Séquard, when removing the adrenal glands of different healthy animals, noticed a drastic reduction in the survival of these animals, concluding that the death resulted from the lack of vital substances (glucocorticoids and catecholamines) produced by the adrenal glands that and released into the current (Aminoff, 1996).

While catecholamines synthesis occurs in the adrenal gland medulla through stimulation of the sympathetic autonomic nervous system, corticoids are released by the adrenal gland cortex under different stimuli, such as physical, emotional, or biochemical stress (Turnbull & Rivier, 1999). Stimulation of the hypothalamus leads to the release of corticotropin-releasing hormone (CRH), produced and released by cells in the paraventricular

nucleus (PVN) (Turnbull & Rivier, 1999). Neurotransmitters released by catecholaminergic or glutamatergic neurons and inflammatory mediators (e.g., cytokines and lipid mediators) induce an increase in intracellular calcium levels in the PVN, promoting the fusion of CRH-containing vesicles to the plasma membrane and the transport of CRH to the pituitary via the portal system (Checkley, 1996). In the pituitary, CRH induces the release of the adrenocorticotropic hormone (ACTH) in the bloodstream, which will induce the synthesis of glucocorticoids by the adrenal glands (Nemeroff, 1996; Guyton & Hall, 2002).

Glucocorticoids are steroid hormones that regulate various physiological responses, such as carbohydrate metabolism and defense responses. (Bavaresco et al., 2005, Caratti et al., 2015). Although circadian synthesis and release occur naturally accordingly to the life habit of each species, factors associated with danger (infections, for example) increase the release of glucocorticoids, which, in turn, modulate the activity of immunocompetent cells (Goodman & Gilman, 2003). Glucocorticoids signalize through two types of intracellular receptors, the mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) (Krozowski & Funder, 1983), which act as transcription factors regulating the programming of cell physiology (Bledsoe et al., 2002; Kumar & Thompson, 2005; Ramamoorthy & Cidlowski, 2013; 2016). Glucocorticoids such as corticosterone (CORT) have a greater affinity for MR than GR (Reul & De Kloet, 1985). Thus, the higher probability of GR activation occurs when glucocorticoid concentration increase after the stimulation of the HPA axis. GR also controls HPA activation

magnitude since these receptors in the hypothalamus decrease ACTH synthesis and reduce adrenal stimulation (Kloet et al., 1998).

Studies by our group show that glucocorticoids have a dual action on melatonin synthesis in the pineal gland (Lopes et al., 1997, 2001; Ferreira et al., 2005, Fernandes et al., 2006, 2009, 2017). Depending on the experimental model, this hormone can potentiate or inhibit melatonin synthesis. (Fernandes et al., 2009). CORT, on the one hand, enhances melatonin production in pineal glands stimulated with low concentrations of NAd (Ferreira et al., 2005; Fernandes et al., 2006). On the other hand, the incubation of the pineal glands with a high dose of CORT (100 µM) and with maximum stimulation of adrenoreceptors inhibits the production of melatonin (Yuwiler, 1989). We clarified part of this dual effect of CORT on melatonin production by the pineal gland. CORT potentiates melatonin production when the pineal gland is under β-adrenergic stimulation but inhibits hormone production when the pineal is stimulated concomitantly with β and α1-adrenergic agonists (Fernandes et al., 2017). In both cases, GR activation and the modulation of the NF-kB pathway are at the basis of the molecular mechanism controlling pineal function (Ferreira et al., 2005; Fernandes et al., 2017). Thus, the context of differential activation of adrenergic receptors is a determining factor in the pattern of response triggered by glucocorticoids on the production of melatonin by the pineal gland.

The proper functioning of immune system cells is essential for organisms to monitor and defend against aggressive events/agents. The anti-inflammatory effect of glucocorticoids is well established and is related to actions on the production of immune mediators by immunocompetent cells.

Interestingly, primary macrophages in culture and RAW 264.7 immortalized linage cells produce melatonin via an NF-kB-dependent pathway, an important event for the phagocytosis process of these cells (Pires-Lapa et al., 2013). Considering that glucocorticoids induce dual effects on melatonin production by mechanisms that involve the GR activation and the inhibition of the NF-kB pathway, it is essential to expand the understanding of this immunoendocrine functional dynamics in immunocompetent cells of lymphoid organs and peripheral tissue-resident macrophages. With these data, we will be able to establish differences and similarities between the effects of corticosterone on cells from different compartments of the organism, thus enabling a broader understanding of the corticoid/melatonin relationship both in healthy situations and in the presence of a defense response.

CONCLUSIONS AND FINAL CONSIDERATIONS

- Corticosterone (1-100 µM) increases the expression of AANAT, PAANAT and ASMT enzymes and, consequently, the production of MEL in RAW 264.7 cells.
- The inhibition of GR impairs the corticosterone effect over AANAT, PAANAT and ASMT in RAW 264.7 cells.
- The expression of AANAT and ASMT in peritoneal phagocytes varies significantly throughout the light/dark phases. The lower expression of the enzymes occurs at the ZT12. The peak of melatonin produced by these cells during the day corresponds to the maximal expression of the enzymes, while the nocturnal peak most probably reflects nocturnal melatonin production.
- Corticosterone and Dexamethasone increase the expression of AANAT and ASMT in peritoneal and bone marrow cells.
- The inhibition of GR or MR reverts the effects suggesting that both receptors are necessary for the corticoid-dependent activation of the melatonergic system in these cells.
- Zymosan increases the expression of the melatonergic enzymes of RAW 264.7, peritoneal and bone marrow cells.
- Corticosterone decreases the zymosan-induced potentiation of the melatonergic system of RAW 264.7, peritoneal and bone marrow cells.
- The blockade of GR or MR does not revert the inhibitory effects imposed by corticosterone upon zymosan-induced activation of the melatonergic system. The blockade of MR even potentiates the inhibitory effect imposed by corticosterone. These data also suggest that both receptors might be involved

in the inhibitory effect of corticosterone over zymosan activation of the melatonergic system.

- GR activated by corticosterone or the antagonist mifepristone inhibits p65 NFκB accumulation induced by zymosan.
- High levels of corticosterone (300 and 1000 μM) decrease the activation of the *Aanat* gene promoter in a mechanism dependent on the mutual antagonism between GR and NFκB.

The present work demonstrates that, similarly to the observed in the pineal gland, corticoids might impose dual effects upon the melatonergic system of immunocompetent cells. The direction of the effect will depend on the dose of corticoid reaching the cells, the activation of GR and MR, and the interaction with pathways triggered by immune-related signals. In our case, we demonstrated that the mutual antagonism between the NFκB pathway (triggered by zymosan) and GR adjusts pineal production by immune cells. In addition, other pathways (STATs, CREB, AP-1) might also influence and be influenced by corticoids in the modulation of the melatonergic system of immune cells, opening an exciting field for future investigations. In this sense, the role of MR upon pineal melatonin production has never been explored and might bring some new interesting insights regarding the interactions of the gland with other hormones, such as aldosterone. Moreover, the results of the present Thesis bring some new information that leads to a better understanding of the immune system' melatonergic system in healthy conditions and the progress of pathophysiologic and pathologic processes.

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CERTIFICADO

Certificamos que a proposta intitulada **"Efeito da corticosterone sobre a produção de melatonina por macrófagos peritoneais"**, registrada com o nº 253/2016 (Proc. 16.1.274.41.8), sob a responsabilidade do Prof. Dr. Pedro Augusto Carlos Magno Fernandes e com a participação da colaboradora Débora dos Santos Silva (IB-USP), que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica encontra-se de acordo com os preceitos da Lei nº 11.794, de 08 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009 e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovada pela Comissão de Ética no Uso de Animais – CEUA do Instituto de Biociências da Universidade de São Paulo, em reunião de 14 de junho de 2016.

Vigência da autorização: 14/06/2016 a 13/06/2017

Finalidade: Pesquisa Científica

Espécies/linhagem: camundongo isogênico/*C57Bl/6 e* Rato isogênico/*Wistar*

Nº de animais: 80

Peso/Idade: 20-40g/2-3 meses e 200-300g/2-3 meses

Sexo: (M)

Origem: Biotério de Roedores do Departamento de Fisiologia do Instituto de Biociências - USP - São Paulo - SP

OBS.: Qualquer alteração e/ou intercorrência deverá ser comunicada a CEUA-IB.

Profa. Dra. Mariz Vainzof

Coordenadora da Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada **"Análise do perfil circadiano do transcriptoma de diversos órgãos e tecidos de ratos saudáveis ou desafiados com doses letais e não letais de LPS"**, registrada com o nº 346/2019, sob a responsabilidade do Prof. Dr. Pedro Augusto Carlos Magno Fernandes e com a participação dos colaboradores, Marlina Olyissa Córdoba Moreno (IB/USP), Gabriela Sarti Kinker (IB/USP) e Regina Pekelmann Markus (IB/USP), que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica encontra-se de acordo com os preceitos da Lei nº 11.794, de 08 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009 e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovada pela Comissão de Ética no Uso de Animais – CEUA do Instituto de Biociências da Universidade de São Paulo, em reunião de 19 de março de 2019.

Vigência da autorização: 19/03/2019 a 01/03/2023

Finalidade: Pesquisa Científica

Espécie/linhagem: Rato heterogênico/*Wistar*

Nº de animais: 1.458(M) **Idade/Peso aprox.:** 2-4meses/250-350g **Total:** 1.458 animais

Origem: Rede de Biotérios da USP, ICB, São Paulo - SP

OBS.: *Qualquer intercorrência ou alteração do projeto em andamento deverá ser previamente autorizada pela Comissão de Ética no Uso de Animais – CEUA-IB.*

Merari F. R. Ferrari

Profa. Dra. Merari de Fátima Ramires Ferrari
Vice-Coordenadora da Comissão de Ética no Uso de Animais