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Progesterone reverts LPS-reduced FAAH activity in murine peripheral blood mononuclear cells by a receptor-mediated fashion

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ABSTRACT

Increased anandamide concentrations are associated with pregnancy failure. Anandamide levels are regulated by the fatty acid amide hydrolase (FAAH). The aim of the study was to investigate the role of progesterone (P) on FAAH modulation in murine peripheral blood mononuclear cells (PBMC) under septic conditions. We observed that *in vivo* administration of LPS to non-pregnant (NP) mice decreased FAAH activity of PBMC while in pregnant mice no changes in FAAH activity were observed. NP animals administered with P had a similar response to LPS as the pregnant animals. Also, NP mice injected with P antagonist and P showed that the effect of P on LPS-reduced FAAH activity was impaired. Furthermore, LPS produced a decrease in the ratio of PR-B/PR-A in NP animals.

Our results showed that, in our model the endotoxin decreased PBMC's FAAH activity and this condition was reverted by P in a receptor-mediated fashion.

1. Introduction

Endocannabinoids are amides, esters, and ethers of long-chain polyunsaturated fatty acids found in several human tissues (Fowler et al., 2001; Hanus et al., 2001). N-arachidonoylethanolamine, anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are the main endocannabinoids described to date (Howlett and Mukhopadhyay, 2000; Sugiura and Waku, 2000). They bind to both brain cannabinoid receptors (CB1 and CB2), which are widely distributed in mammalian tissues (Sun and Dey, 2012) and their effects are terminated by their rapid uptake and subsequent intracellular degradation. Strong genetic and pharmacological evidence has demonstrated that fatty acid amide hydrolase (FAAH) inactivates AEA (Cravatt et al., 2001; Kathuria et al., 2003; Maccarroneet al., 2010). On the other hand, it has been reported that 2-AG is inactivated by monoacylglycerol lipase (MAGL) (Dinh et al., 2002; Bisogno et al., 1997, 2001; Beltramo and Piomelli, 2000). However, when MAGL protein is fully knocked down by RNA interference (RNAi), 50% of the 2-AG hydrolyzing activity remains in cell homogenates, indicating that additional enzymes may hydrolyze this lipid (Dinh et al., 2004). Prime candidates are FAAH and the cyclooxygenases (Kozak et al., 2000; Goparaju et al., 1998).

Anandamide and 2-AG are two of the best-studied members of the endocannabinoid family and they have been described as the major endocannabinoids present in the uterus suggesting that they might play a role in reproduction (Wang et al., 2007). The endogenous tone of AEA is the checkpoint for the regulation of its action. Evidence has been provided that low levels of AEA are favorable for implantation and trophoblast outgrowth while increased AEA concentrations are associated with retarded embryo development, fetal loss and pregnancy failure (Paria and Dey, 2000). Cravatt and Lichtman (2003) suggested that both *in vivo* AEA "tone" and biological activity are regulated by FAAH. A similar pattern of 2-AG was noted in the uterus during early pregnancy (Wang et al., 2007). Moreover, it has been reported that high plasma AEA levels are associated with early pregnancy loss in humans (Habayeb et al., 2008). In fact, in women undergoing *in vitro* fertilization and

Abbreviations: AEA, anandamide; 2-AG, 2-arachidonoylglycerol; CB, cannabinoid receptor EmRe: embryonic resorption FAAH: fatty acid amide hydrolase; LPS, lipopolysaccharide; LONA, Lonaprisan; NAPE-PLD, N-arachidonoyl phosphatidylethanolamine; MAGL, monoacylglycerol lipase; PBMC, peripheral blood mononuclear cells; P, progesterone; PR, progesterone receptor.

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embryo transfer, high plasma AEA levels and low lymphocyte FAAH levels at 6 weeks after embryo transfer were associated with failure to achieve an ongoing pregnancy (Maccarrone et al., 2002). It has been suggested that FAAH present in peripheral T cells has a crucial role in controlling pregnancy (Maccarrone and Finazzi-Agrò, 2004; Bambang et al., 2012). Also, it was demonstrated recently that peripheral FAAH activity is significantly reduced in ectopic pregnancies (Gebeh et al., 2013).

Progesterone (P), a hormone essential not only for the establishment but also for the maintenance of pregnancy, is also known to modulate immune function (Correale et al., 1998) and to elicit an immunological response critical for normal gestation (Szekeres-Bartho et al., 1996). The need for progesterone is shown by the fact that blocking progesterone binding sites causes abortion or preterm labor in humans and various animal species (Elger et al., 1987; Winer et al., 2009). It has been reported that P upregulates FAAH activity in human lymphocytes through up-regulation of gene expression at transcriptional and translational level (Maccarrone et al., 2001a).

On the other hand, genital tract infections caused by Gram-negative bacteria induce miscarriage and are one of the most common complications of human pregnancy (Cram et al., 2002). Our previous results show that exposure of pregnant female mice (through i.p. injections) to lipopolysaccharide (LPS), a component of the cell walls of Gram-negative bacteria, leads to embryonic resorption (EmRe) followed by fetal expulsion (Ogando et al., 2003). Histological analysis of implantation sites from pregnant female mice challenged *in vivo* with LPS showed that the part of the decidua closer to the uterus was highly infiltrated by granulocytes and LGL cells (large granular lymphocytes) (Ogando et al., 2003). Moreover, LPS-induced EmRe is associated with an increased uterine and decidual production of nitric oxide (NO) and prostaglandins (PGs), two molecules that play key roles in this process (Ogando et al., 2003; Aisemberg et al., 2007). Furthermore, our previous work suggests that LPS could augment AEA levels in uterine explants by inhibiting its degradation Indeed, we demonstrated that AEA regulate LPS-induced NO production and tissue damage in the uterus of pregnant mice (Vercelli et al., 2009).

In addition, Maccarrone et al. (2001b) showed that LPS downregulates FAAH expression and increases AEA levels in human peripheral lymphocytes while Liu et al. showed that LPS induces AEA but not 2-AG in murine macrophages (Liu et al., 2003).

Taking into consideration that: (1) in our model of LPS-induced EmRe the decidua was highly infiltrated by granulocytes and LGL cells, (2) that LPS and P regulate endocannabinoid metabolism in human T lymphocytes and, (3) that high levels of endocannabinoids are associated with early pregnancy loss, our aim was therefore to investigate the role of P on FAAH modulation in murine peripheral blood mononuclear cells (PBMC) in our model of LPS-induced EmRe and to determine which P receptors if any were involved in this effect.

2. Materials and methods

2.1. Reagents

LPS from Escherichia coli 05:B55, anti-β-actin antibody and progesterone, were purchased from Sigma Chemical Co. (St Louis, MI, USA), [^{3}H]-anandamide (specific activity 172.4 Ci mmol^{-1}) was provided by Perkin Elmer (Boston, MA, USA). Thin Layer Chromatography (TLC) aluminum Silica Gel plates were purchased from Merk KGaA (Darmstadt, Germany). The western blotting reagents were obtained from Bio-Rad (Tecnolab, Argentina). Secondary horse radish peroxidase (HRP) conjugated antibody was purchased from Jackson Immunosearch (Baltimor Pike, USA). The anti-FAAH antibody was a gift from Dr. Benjamin Cravatt (Scripps, La Joya, San Diego USA). The anti-PR (C-19) rabbit polyclonal antibody was provided by Santa Cruz biotechnology (Tecnolab, Argentina). Anandamide and RU-486 were purchased from Biomol (Enzo Life Sciences, Miami, FL, USA).As RU486 is also an antagonist of glucocorticoid receptors, we decided to use the more specific and potent progesterone antagonist, Lonaprisan (ZK-230211) (Fuhrmann et al., 2000; Afhüppe et al., 2009), which was kindly provided by Bayer-Schering (Germany). Trizol reagent, RNAse-free DNAse I, Moloney Murine Leukemia virus reverse transcriptase (M-MLVRT) and random primers were purchased from Invitrogen (Life Technologies, Argentina). GoTaq DNA Polymerase was purchased from Promega (Biodynamics, Argentina). All other chemicals were analytical grade.

2.2. Animals and treatments

8- to 12-week-old virgin female BALB/c mice were paired with 8- to 12-week-old BALB/c males and the day of appearance of a coital plug was taken as day 0 of pregnancy. Animals were housed in cages under controlled conditions of light (14 h light, 10 h dark) and temperature (23–25 °C) and received murine chow and water ad libitum.

Non-pregnant mice were divided into six groups (Supplementary Fig. 1A): (i) *control*: females received an i.p. injection of vehicle and 14 h later were administered another i.p. injection of vehicle; (ii) *RU486/Lonaprisan/progesterone*: females received an i.p. injection of RU486 (10 μg g^{-1} of body weight) or Lonaprizan (LONA. 1 μg g^{-1} of body weight in 1:2 EtOH:NaCl 0.9%) or progesterone (P, subcutaneous (s.c.), 4 μg g^{-1} of body weight in oil) and 14 h later were administered another dose of RU486 or LONA or P; (iii) *LPS*: females received an i.p. injection of vehicle and 14 h later were administered an i.p. injection of LPS (1 μg g^{-1} of body weight); (iv) *LPS plus P*: females received a s.c. injection of P and 14 h later were administered an i.p. injection of LPS plus another s.c. injection of P; (v) *LPS plus P plus RU486*: females received a s.c. injection of P plus an i.p. injection of RU486. Fourteen hours later females were administered an i.p. injection of LPS plus another s.c. injection of P and another i.p. injection of RU486; (vi) *LPS plus P plus LONA*: females received a s.c. injection of P plus an i.p. injection of LONA. Fourteen hours later females were administered an i.p. injection of LPS plus another s.c. injection of P and another i.p. injection of LONA. Animals were euthanized 6 h after LPS administration.

Pregnant mice were divided into four groups (Supplementary Fig. 1B). On day 7 of pregnancy: (i) *control:* females received an i.p. injection of vehicle and 2 h later were administered another i.p. injection of vehicle; (ii) *LPS:* females received an intraperitoneal (i.p.) injection of vehicle and 2 h later were administered an i.p. injection of LPS (1 μg g^{-1} of body weight); (iii) *RU486*: females received an i.p. injection of RU486 (10 μg g^{-1} of body weight) and 2 h later were administered an i.p. injection of vehicle; (iv) *LPS plus RU486*: females received an i.p. injection of RU486 and 2 h later were administered an i.p. injection of LPS. Animals were euthanized 6 h after LPS or vehicle administration.

Note: administration of RU486 to pregnant animals produced embryonic resorption and fetal expulsion within a time frame of 12 h. Due to this, the dosing schedule was reduced from a 14 h to a 2 h time frame.

2.3. Ethics statement

The experimental procedures reported here were approved by the Animal Care Committee of the Center for Pharmacological and Botanical Studies of the National Research Council (CEFYBO – CONICET) and by The Institutional Committed for the Care and Use of Laboratory animals from the School of Medicine (University