Mouse blastocysts release a lipid which activates anandamide hydrolase in intact uterus

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Anandamide (N-arachidonylethanolamine, AEA) is a major endocannabinoid, known to impair mouse pregnancy and embryo development and to induce apoptosis in blastocysts. Here we show that mouse blastocysts rapidly (within 30 min of culture) release a soluble compound, that increases by ~2.5-fold the activity of AEA hydrolase (fatty acid amide hydrolase, FAAH) present in the mouse uterus, without affecting FAAH gene expression at the translational level. This ‘FAAH activator’ was produced by both trophectoderm and inner cell mass cells, and its initial biochemical characterization showed that it was fully neutralized by adding lipase to the blastocyst-conditioned medium (BCM), and was potentiated by adding trypsin to BCM. Other proteases, phospholipases A2, C or D or DNase I or RNase A were ineffective. BCM did not affect the AEA-synthesizing phospholipase D, the AEA-binding cannabionid receptors, or the selective FAAH membrane transporter in mouse uterus. The FAAH activator was absent in uterine fluid from pregnant mice and could not be identified with any factor known to be released by blastocysts. In fact, platelet-activating factor inhibited non-competitively FAAH in mouse uterus extracts, but not in intact uterus horns, whereas leukotriene B4 or prostaglandins E2 and F2α had no effect. Overall, it can be suggested that blastocysts may protect themselves against the noxious effects of uterine endocannabinoids by locally releasing a lipid able to cross the cell membranes and to activate FAAH. The precise molecular identity of this activator, the first ever reported for FAAH, remains to be elucidated.

Key words: anandamide/enzyme activation/fertility/hydrolysis/implantation

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), and in human reproductive fluids (Schuel et al., 2002). Anandamide (N-arachidonylethanolamine, AEA) and 2-arachidonoylglycerol are the main endocannabinoids described to date (Howlett and Mukhopadhyay, 2000). They bind to both type-1 (CB1) and type-2 (CB2) cannabinoid receptors, thus mimicking several central and peripheral effects of Δ9-tetrahydrocannabinol (THC), the main active ingredient of hashish and marijuana (Mechoulam and Hanus, 2000). At the periphery, AEA and 2-arachidonoylglycerol show cardiovascular (Kunos et al., 2000), immune (Parolaro et al., 2002) and anti-inflammatory activities (De Petrocellis et al., 2000). Moreover, endogenous cannabinoids have been involved in the inhibition of human breast and prostate cancer cell proliferation (Melck et al., 2000). The effect of AEA via CB1 and CB2 receptors depends on its extracellular concentration, which is controlled by (i) cellular uptake by a specific AEA membrane transporter (AMT), and (ii) intracellular degradation by the AEA-hydrolysing enzyme fatty acid amide hydrolase (FAAH). AMT (Hillard and Jarrahian, 2000) and FAAH (Ueda et al., 2000) have been characterized in several mammalian cells and tissues, and together with AEA and congeners form the ‘endocannabinoid system’.

Evidence is accumulating about endocannabinoid modulation of embryo–uterine interactions (Paria and Dey, 2000), and impairment of pregnancy and embryo development in mice (Yang et al., 1996), thus recalling the adverse effects of THC on reproduction (Hall and Solowij, 1998). More recently, progesterone has been shown to be involved in THC modulation of sexual receptivity in female rats (Mani et al., 2001), and dysregulation of cannabinoid signalling has been shown to disrupt uterus receptivity to embryo implantation in mice (Paria et al., 2001). Along this line, we reported the association between decreased FAAH activity and expression in maternal peripheral lymphocytes and early pregnancy failure in humans, demonstrating that an impairment of AEA degradation might be connected with reduced fertility (Maccarrone et al., 2000a). Consistently, down-regulation of AEA levels in mouse uterus has been associated with increased uterine receptivity, which instead decreases when AEA is up-regulated (Yang et al., 1996; Schmid et al., 1997). Mouse uterus contains the highest levels of AEA so far detected in mammalian tissues, and is the only tissue where AEA appears to be the main (up to 95%) N-acylthanolamine (Schmid et al., 1997). Furthermore, the developmental arrest that occurs in mouse blastocysts in a non-receptive uterine environment correlates well with its higher levels of AEA and with the in vitro observation that AEA inhibits embryo development and zona-hatching of blastocysts (Paria et al., 1996; Yang et al., 1996; Schmid et al., 1997).

Mouse blastocysts express a biologically active CB1 receptor, together with specific AMT and FAAH (Maccarrone et al., 2000b). They are driven to apoptosis by AEA, suggesting that an efficient
degradation of this compound might be instrumental in preventing its blastotoxic effects (Maccarrone et al., 2000b). As a matter of fact, recent observations on FAAH mRNA accumulation (Paria et al., 1999) and FAAH activity (Maccarrone et al., 2000b) in pregnant mice suggest that this enzyme may be the ‘checkpoint’ for maintaining the steady levels of AEA. Indeed, FAAH activity has recently been shown to correlate inversely with AEA levels in various tissues of FAAH knockout mice versus wild-type animals (Cravatt et al., 2001) and in blood of women with normal gestation versus those who aborted (Maccarrone et al., 2002). Here, we report that blastocysts release a soluble factor able to actuate uterine FAAH, thus reducing the level of AEA and its negative effects on blastocyst survival and implantation.

Materials and methods

Materials and enzymes

All chemicals were of the purest analytical grade. Leukotriene B4 (LTB4) and prostaglandins E2 (PGE2) and F2α (PGF2α) were from Cayman Chemical Company (USA). Platelet activating factor-16 (1-O-palmitoyl-2-acetyl-sn-glycero-3-phosphocholine; PAF-16) and -18 (1-O-stearyl-2-acetyl-sn-glycero-3-phosphocholine; PAF-18) were obtained from Genzyme (Cambridge, MA, USA). 

1,2-Dioleoyl-3-phosphatidyl[2-14C]ethanolamine (55 mCi/mmol) and [3H]arachidonic acid ([3H]AA) and [3H]methanethiol ([3H]MT) were obtained from NEN DuPont de Nemours (Boston, MA, USA). 126 Ci/mmol) was from New England Nuclear (Boston, MA, USA). 

Uterine horns were excised from virginal female mice at a random stage of the estrous cycle. Anti-FAAH polyclonal antibodies were raised in rabbits against the conserved FAAH sequence VGYETDNTPMFAMR (Giang and Cravatt, 1997) conjugated to ovalbumin, and were prepared by Pirm S.r.l. (Italy). Goat anti-rabbit antibodies conjugated to alkaline phosphatase (GAR-AP) were from Bio-Rad (USA).

Blastocyst culture and collection of conditioned medium

CD-1 mice were purchased from Charles River (Italy). Uterine horns were excised from virgin female mice at a random stage of the estrous cycle as reported (Maccarrone et al., 2000b), and early blastocysts (3.5 days post coitum) were collected in the mid morning by flushing the uterus with M2 medium plus 1 mg/ml polyvinylpyrrolidone (Sigma) (Maccarrone et al., 2000b). Fifty-five blastocysts were then cultured in 20 ml M16 medium in Terasaki’s 96 microwells for the indicated periods of time, at 37°C and 5% CO2 in humidified incubator. At the end of the incubation, blastocysts were removed and the blastocyst-conditioned medium (BCM) was used as such for subsequent analysis. In some experiments, blastocysts were killed by rapid freezing (–80°C) and thawing (37°C) and were then kept at 37°C in M16 medium for 12 h. In other experiments, morulae (2.5 days post coitum) or late blastocysts (4.5 days post coitum) were used to produce conditioned media (CM) with the same procedure described above (25 morulae or late blastocysts in 20 ml M16 medium for the indicated periods, at 37°C). Morulae and late blastocysts were collected by flushing the oviduct and uterus respectively. In yet other experiments, blastocysts were obtained following in vitro culture of 2-cell embryos (2-CE) in 0.5 ml M16 medium plus 4 mg/ml bovine serum albumin (fraction V; Sigma) in a well of a 4-well Nuncolon dish (Nunc, Denmark) for 4 days at 37°C in 5% CO2. At the end of the culture period, 2-CE-derived blastocysts were washed and cultured for 2 h in M16 medium in Terasaki’s microwells, in order to prepare CM as described above. With the aim of ascertaining the source of the FAAH activator in the blastocyst, trophoblast and inner cell mass (ICM) cells were separated as follows. Briefly, 25 early blastocysts were cultured in 20 μl M16 + 10% FCS (Life Technologies) for 5 days in Terasaki’s microwells at 37°C in 5% CO2. Under these conditions, trophoblast cells attach and spread onto the culture dish, forming a monolayer, whereas ICM cells remain on top of the trophoblast cells. Using a mouth-operated glass micropipette, ICM cells were displaced from the trophoblast cell monolayer and transferred to another well. CM was prepared by incubating 25 trophoblasts or 25 ICM cells in 20 μl M16 for 2 h as reported above. Finally, the presence of the FAAH activator was analysed in uterine fluids and in STO cell monolayers (mouse embryonic fibroblasts, from American Tissue and Cell Collection, UK). Uterine fluids were obtained by flushing uterine horns of pregnant CD-1 mice at 3.5 days post coitum with ~100 μl of M16 per horn. STO cells were grown in DMEM supplemented with 10% FCS under standard culture conditions. CM was prepared by incubating STO cells (~8×105 cells/ml) in M16 as described above.

The effect of various enzymes on BCM was assessed by incubating the conditioned medium with each enzyme (10 IU) for 1 h at 37°C, before using BCM in FAAH activity assays (see below). All procedures were approved by the Animal Care Committee of University of Teramo and University of Rome ‘Tor Vergata’.

Assay of FAAH activity and expression

Fatty acid amide hydrolase (EC 3.5.1.14; FAAH) activity in extracts of mouse uterine horns was determined by reversed-phase high performance liquid chromatography, following the release of [3H]arachidonic acid from 10 μmol/l [3H]AEA at pH 9.0 as reported (Maccarrone et al., 1999). Tissues were homogenized by UltraTurrax T25 in 50 mmol/l Tris–HCl and 1 mmol/l EDTA, pH 7.4, at a 1:10 homogenization ratio (fresh weight/volume), then the homogenate was centrifuged sequentially at 800 g for 5 min and 11 000 g for 20 min at 4°C (Maccarrone et al., 1999). The final pellet was resuspended in ice-cold buffer at a protein concentration of 1 mg/ml and stored at –80°C until use. FAAH activity was assayed in uterine extracts in the presence of BCM (or plain medium in the controls), or of various compounds added directly to the assay buffer (100 μl). In the case of PAF-16 and PAF-18, the inhibition constant (Ki) of FAAH-catalysed hydrolysis of [3H]AEA (in the range 0–25 μmol/l) was determined by Lineweaver–Burk analysis of the experimental data (Maccarrone et al., 1999). FAAH activity was also assayed in intact uterine horns excised from virginal female mice at a random stage of the estrous cycle. Horns (2/test) were pre-incubated at 37°C for 2 h in phosphate-buffered saline (100 μl), containing BCM (or plain medium in the controls) or other compounds, and then washed and homogenized in assay buffer for subsequent analysis of FAAH activity and expression. For each animal, one horn was treated with BCM and the other one with plain medium as a control.

The FAAH protein content in uterine horns was assessed by enzyme-linked immunosorbent assay (ELISA), as reported (Maccarrone et al., 2001). Wells were coated with tissue homogenates (20 μg of proteins/well) prepared as described above for FAAH assay. Wells were then reacted with anti-FAAH antibodies (diluted 1:500), using GAR-AP (diluted 1:2000) as secondary antibody (Maccarrone et al., 2001).

Determination of AMT activity, CBR binding and PLD activity in mouse uterus

The uptake of 400 nmol/l [3H]AEA by the AEA membrane transporter (AMT) of synaptosome-like vesicles prepared from uterine horns was determined as described (Battista et al., 2002). To discriminate non-carrier-mediated from carrier-mediated transport of AEA through cell membranes, [3H]AEA uptake at 4°C was subtracted from that at 37°C (Hillard et al., 1997).

For cannabinoid receptor studies, membrane fractions were prepared from fresh uterine horns as reported (Maccarrone et al., 2000c) and immediately used in rapid filtration assays with the synthetic cannabinoid [3H]CP55,940 at 400 pmol/l (Maccarrone et al., 2000c). Non-specific binding was assessed in the presence of 10 μmol/l AEA (Deutsch et al., 1997).

The activity of phospholipase D (EC 3.1.4.4; PLD) was assayed in homogenates of uterine horns by measuring the release of [14C]ethanolamine.
from 1,2-dioleoyl-3-phosphatidyl-[2-14C]ethanolamine (10 μmol/l), as described (Gubellini et al., 2002).

Statistical analysis
Data reported in this paper are the mean (± SD) of at least three independent determinations, each in duplicate. Statistical analysis was performed by the non-parametric Mann–Whitney test, analysing experimental data by means of the InStat 3 program (GraphPAD Software for Science, USA).

Results

BCM activates uterine FAAH
The addition of blastocyst-conditioned medium (BCM), i.e. medium in which early blastocysts had been cultured for 2 h, to extracts of mouse uteri enhanced FAAH activity in a dose-dependent manner, while plain medium had no effect (Figure 1A and data not shown). In addition, BCM (up to 10 μl) did not show any ability to hydrolyse AEA, ruling out that it might contain FAAH activity. A dose-dependent, yet less pronounced, activation of FAAH was also observed when intact uterine horns were preincubated for 2 h in BCM, and then assayed for FAAH activity under the same experimental conditions (Figure 1A). In both cases, FAAH activation reached statistical significance (P < 0.05) at 2 μl BCM and a maximum at 4 μl BCM. Therefore, the latter amount was chosen to further investigate the effect of BCM on FAAH.

The ability of BCM to activate FAAH, both in uterine homogenates and intact uterine horns, depended on the duration of the preincubation of blastocysts in culture medium, reaching statistical significance (P < 0.05) at 30 min and a maximum at 2 h, with no further increase in the following 10 h (Figure 1B). Therefore, BCM in which blastocysts had been cultured for 2 h was used in all further analyses, unless stated otherwise. The medium in which dead blastocysts had been kept for 12 h was unable to enhance FAAH activity (Figure 1B). CM obtained from morulae or late blastocysts enhanced FAAH activity in uterine extracts up to 150 and 330% of the controls, compared to the 250% increase induced by BCM (shown in Figure 1A). The difference between the effect of BCM and that of the medium conditioned with morulae or with late blastocysts was statistically significant (P < 0.05 in both cases). CM from blastocysts derived from 2-CE showed a similar ability to enhance FAAH in uterine extracts compared to CM from freshly collected blastocysts (200 versus 250% over the untreated controls respectively).

Western blot analyses of extracts of mouse uterus had shown that specific anti-FAAH antibodies recognized a single immunoreactive band of the molecular size expected for FAAH (Maccarrone et al., 2000b). Anti-FAAH antibodies were used here to quantify FAAH content by ELISA, which showed that enhancement of FAAH activity by BCM was not paralleled by an increase of FAAH protein (Table I). Accordingly, enzyme activity was almost abolished by 100 nmol/l MAFP, a selective and powerful irreversible inhibitor of FAAH (Gubellini et al., 2002), in both BCM-treated and control mouse uterus.

Figure 1. Effect of blastocyst-conditioned medium (BCM) on fatty acid amide hydrolase (FAAH) activity in mouse uterus. (A) FAAH activity in uterine tissue homogenates that had been exposed to various volumes of blastocyst-conditioned medium (25 blastocysts/20 μl per test, 37°C for 2 h) before or after homogenization, and referred to as intact tissue or homogenate respectively. (B) Dependence of FAAH activity in the same uterine tissue homogenates on the duration of the pre-incubation of blastocysts in culture medium (25 blastocysts/20 μl per test). In each assay, 4 μl BCM was used. (C) Effect of various enzymes (10 IU each) on the ability of 4 μl BCM in which blastocysts had been cultured for 2 h to enhance FAAH activity in tissue homogenates or in intact horns. The proteases α-chymotrypsin, bromelain, papain and pepsin A (10 IU each) had no effect, and were omitted for the sake of clarity. In all panels, 100% = 170 ± 18 pmol/min/mg of protein. (A, B) *P < 0.05, **P < 0.01 versus controls (P > 0.05 in all other cases). (C) *P < 0.05, **P < 0.01 versus BCM-treated samples (P > 0.05 in all other cases).
without affecting the corresponding protein level, whereas the protein synthesis inhibitor cycloheximide (10 µg/ml) affected neither FAAH activity nor protein content (Table I).

In order to determine whether the FAAH activator was produced by trophoblast or ICM cells, we separated trophoblast from ICM of early blastocysts. The results showed that CM obtained from trophoblast and ICM cells possess the ability to enhance FAAH activity (Table II). Interestingly, the ability of CM from trophoblast cells to enhance FAAH activity was ~2-fold and ~2.5-fold higher than that of blastocysts or ICM cells respectively. Finally, we found that uterine fluid did not contain any FAAH activator, neither did CM obtained from STO cell monolayers (Table II).

**Table II. Effect of trophoblast and inner cell mass (ICM) cells on fatty acid amide hydrolase (FAAH) activity**

<table>
<thead>
<tr>
<th>Conditioned medium added (4 µl/100 µl)</th>
<th>FAAH activity*</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>170 ± 18 (100)</td>
</tr>
<tr>
<td>Trophoblast-derived cells</td>
<td>785 ± 75 (462)*</td>
</tr>
<tr>
<td>ICM cells</td>
<td>301 ± 30 (177)*</td>
</tr>
<tr>
<td>Uterine fluid</td>
<td>152 ± 16 (89)</td>
</tr>
<tr>
<td>STO cells</td>
<td>153 ± 16 (90)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages. The effect of conditioned media in which different cell types had been cultured for 2 h was assayed on mouse uterine FAAH. Also the effect of uterine fluid from pregnant mice (4 µl/100 µl) and of embryonic fibroblasts (STO cells) was assessed.


digestions of BCM and that of various factors known to be released by blastocysts (Psychoyos et al., 1995; Ammit and O’Neill, 1997; Ahmed et al., 1998) on uterine FAAH activity. Treatment of BCM with DNase I, RNase A, phospholipases (PL) A2, C, and D, α-chymotrypsin, bromelain, papain or pepsin A (10 IU each) did not affect its ability to enhance FAAH, either in tissue homogenates or in intact uterine horns, whereas trypsin (10 IU) further increased and lipase (10 IU) almost completely abolished such activity (Figure 1C). Treatment of uterine extracts or intact horns with trypsin or lipase did not affect FAAH activity under the same experimental conditions (not shown).

Platelet-activating factors (PAF)-16 and -18 were found to inhibit FAAH in tissue homogenates in a dose-dependent manner. Kinetic analysis of the inhibition indicated that PAF-16 and -18 acted as non-competitive inhibitors of FAAH, with Ki of ~3 µmol/l and ~2 µmol/l respectively (Figure 2A, and data not shown). Remarkably, FAAH activity of intact uterine horns was not affected by PAF-16 or -18 under the same experimental conditions (Figure 2B). Furthermore, none of the blastocyst-derived eicosanoids, i.e. leukotriene B4 or prostaglandins E2 and F2α, affected FAAH activity in uterine homogenates or intact tissues, when used at concentrations as high as 1 µmol/l (Figure 2B).

**Discussion**

Here, we have shown that mouse blastocysts rapidly (within 30 min of culture) release (a) compound(s), which increase(s) by ~2.5-fold the activity, but not the expression, of FAAH in mouse uterus. This ‘FAAH activator’ does not affect other proteins of the endocannabinoid system, and cannot be identified with any compound known to be released by blastocysts. It can be suggested that this activator may be instrumental in protecting blastocysts against the noxious effects of uterine endocannabinoids during implantation. The observation that only live blastocysts can release the activator of FAAH (Figure 1B), while embryonic fibroblasts (STO cells) cannot (Table II), seems to support the hypothesis of a specific cross-talk between blastocyst and receptive uterus, affecting the level of FAAH. The release of the activator, besides being specific for blastocysts, seems to correlate with the competence of these cells to implant: late blastocysts > early blastocysts > morulae. In particular, the ability to produce the FAAH activator develops in the blastocyst independently of the influence of the uterine environment, as shown by the finding that blastocysts isolated from the uterus or obtained in culture from 2-cell embryos have a similar ability to secrete this compound in the medium. Interestingly, both ICM cells and trophoblasts (each used at 25/well), were able to release in vitro the FAAH activator, while the
contribution of uterus-derived factor(s) could be ruled out. Trophoblast cells appear to possess the greatest ability to produce the FAAH activator, in keeping with their critical role in embryo implantation. On the other hand, the lack of FAAH activator in uterine fluid of pregnant female may indicate that this compound is diluted in such a milieu or during flushing. At any rate, it demonstrates that the activator present in BCM derives from blastocysts, which are likely to be the major source of this substance. Moreover, these findings suggest that the action of such an activator may be important locally at the blastocyst implantation site rather than in the rest of the uterus.

Successful implantation is the result of an intimate cross-talk between the blastocyst and a receptive uterus (Paria et al., 1993). AEA might be critical in regulating the ‘window’ of implantation by synchronizing trophoblast differentiation and uterine preparation to the receptive state. This hypothesis is consistent with the observation that low levels of cannabinoid receptor agonists accelerate trophoblast differentiation and outgrowth, while higher doses inhibit trophoblast differentiation (Wang et al., 1999). Along these lines, higher levels of AEA found in non-receptive uterus correlate well with the developmental arrest, as well as with the observation that in vitro AEA inhibits embryo development and zona-hatching of blastocysts (Paria et al., 1996; Yang et al., 1996; Schmid et al., 1997) and induces apoptosis in these embryos (Maccarrone et al., 2000b). While mouse blastocysts have active AMT and FAAH, suggesting that AEA degradation might be instrumental in preventing its noxious effects on developing embryos (Maccarrone et al., 2000b), mouse uterus contains the highest levels of AEA detected in mammalian tissues to date (Schmid et al., 1997). Therefore, it might be advantageous to the embryo to reduce AEA levels locally by stimulating uterine FAAH, which is confined to the epithelium (Maccarrone et al., 2000b). To date, a FAAH activator has never been reported. Of course, a suitable ‘activator’ of uterine FAAH should be rapid, selective towards its target enzyme and be able to reach the inside intact cells, by crossing easily the plasma membrane barrier. The blastocyst-derived ‘FAAH activator’ described here appears to fulfill these requirements (Figure 1A–C and Table III). A lipid nature of this activator, suggested by the neutralizing effect of lipase (Figure 1C) and by the lack of effect on FAAH expression (Table I), seems to require that the activator interacts directly with the enzyme. As a matter of fact, a direct interaction is likely to occur between a lipid and a lipid-degrading enzyme. Moreover, it seems compatible with the potentiating effect of trypsin on the ability of BCM to enhance FAAH (Figure 1C). In fact, it is conceivable that a lipid compound released into an aqueous solution...
needs a protein carrier, which might be cleaved at a specific site by trypsin, thus releasing higher amounts of activator, and/or possibly in a faster manner. In addition, the data on intact uteri suggest that the activator has the unprecedented ability to diffuse through the myometrium and endometrial stroma to reach the luminal epithelium where FAAH is localized, and to remain bound to the enzyme during processing of the tissue. Also these features seem to speak in favour of a lipid nature of the activator, suggesting that it may be retained into a lipid-binding pocket of FAAH.

In an attempt to identify the FAAH activator released by blastocysts, we checked the effect on FAAH of signals known to be released by blastocysts. They include PAF, leukotrienes and prostaglandins (Psychoyos et al., 1995; Ammit and O’Neill, 1997; Ahmed et al., 1998), which may act as triggers of signal transduction pathways (Dean et al., 2000) and as controllers of blastocyst transport to the uterus (Velasquez et al., 2001) in humans. However, none of these compounds was able to activate mouse uterine FAAH (Figure 2). Two types of PAF, containing either palmitate (PAF-16) or stearate (PAF-18), were non-competitive inhibitors of FAAH in homogenates but not in intact uteri (Figure 2A), thus suggesting that the signalling pathways triggered by the binding of PAF to its receptor (Ammit and O’Neill, 1997; Ahmed et al., 1998; Dean et al., 2000) did not affect FAAH. The possible significance of this effect on FAAH, and hence on AEA homeostasis, in pathophysiological conditions remains to be elucidated. It should be pointed out that blastocyst-conditioned medium is only available in tiny amounts, far below the quantities needed for the biochemical purification of the FAAH activator. Despite the incomplete characterization, the release by blastocysts of (a) compound(s) able to enhance FAAH activity in intact uterine cells appears to be a very interesting outcome of this investigation, which further strengthens the concept that the preimplantation mammalian embryo produces factors which can influence its own development (Lane and Gardner, 1992). In this context, the critical role of FAAH in the cross-talk between blastocyst and receptive uterus described in this study seems to hold true for mice with a normal genetic background, but apparently not for those lacking the FAAH gene. In fact, the latter animals are fertile, and are indistinguishable from wild-type but apparently not for those lacking the gene. In fact, the latter animals are fertile, and are indistinguishable from wild-type

References


high anandamide levels are associated with failure to achieve an ongoing pregnancy after IVF and embryo transfer. Mol Hum Reprod 8,188–195.


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