# CANNABINOID – INDUCED CHANGES IN RAT UTERINE CONTRACTILITY

*Ali K. Asala' akmal A. Diab, Kamelia I. Atia and Maha A. Hamid*
*Physiology department, Faculty of medicine, Zagazig University*

## ABSTRACT

***Background:*** Endocannabinoids are endogenous ligands for cannabinoid receptors. They have been demonstrated in many mammalian tissues and are widely distributed in the CNS, peripheral nerves, leukocytes, spleen and testicles. The uterus contains the highest levels of anandamide; the first discovered Endocannabinoid, suggesting a particular role of anandamide in female reproductive system.

***Objective:*** The present study was designed to demonstrate the effect of anandamide on spontaneous contraction of pregnant and non pregnant rat uterus and to investigate the possible involvement of cannabinoid CB1 receptors, Nitric Oxide (NO), and small conductance $Ca^{+2}$ activated $K^+$ channels in anandamide induced effect.

***Materials & Methods:*** The present study was carried out on a total number of 30 adult albino rats (24 females and 6 males). The male rats were used for induction of pregnancy. The first day of pregnancy was determined by the presence spermatozoa in the vaginal smear examined microscopically. The female rats were divided into four equal groups each contains 6 rats. Three groups (non pregnant, day 10 and day 19 of gestation) were used to study the effects of anandamide ($10^{-6}$, $10^{-5}$ and $10^{-4}$ M/ml organ bath fluid) on spontaneous contractile activity of isolated uterine strips. The fourth group (day 10 of gestation) was used to study the possible mechanisms of action of anandamide using CB (1) receptor antagonist (AM251, $10^{-6}$ M/L), $N^G$-nitro-L-arginine methyl ester (L-NAME, 3 x $10^{-5}$ M/L), and small conductance $Ca^{++}$ activated $K^+$ channels Blocker (Apamin, $10^{-8}$ M/L).

***Results:*** The present study showed that anandamide exerted a significant dose dependant reduction in frequency and amplitude of spontaneous contraction of uterine strips isolated from both pregnant and non pregnant rats. After incubation of uterine strips isolated from pregnant rats on day 10 of gestation with the specific $CB_1$ receptor antagonist AM251, the utero-relaxant effect of anandamide was almost completely abolished. This finding indicates that CB1 receptors are present in the rat uterus and may be the main receptor subtype involved in endocannabinoid-induced uterine relaxation. It was also found that pretreatment of uterine strips with NO synthase inhibitor (L-NAME) and small conductance-$Ca^{+2}$ activated $K^+$ channel blocker (Apamin) significantly decreased the anandamide induced utero-relaxant effect. The utero-relaxant effect of anandamide was significantly more potent in uterine strips isolated from pregnant rats on day 10 of gestation than that in both non pregnant rats and pregnant rats on day 19 of gestation.

***Conclusion:*** Anandamide exerts a potent relaxant effect in vitro on uterine smooth muscles isolated from pregnant and non pregnant rat uterus. This relaxant effect is higher in mid-gestation and this may help uterine quiescence during pregnancy, then diminishes in late pregnancy which may allow effective uterine contraction to occur during labor. The direct relaxant effect of anandamide is mediated through binding with CB1 receptors. Moreover, activation of nitric oxide generation and opening of small conductance $Ca^{++}$ activated $K^+$ channels play a role in this anadamide induced utero-relaxant effect.

## INTRODUCTION

Over the past two decades a number of endogenous compounds that act as ligands for the cannabinoid receptors have been discovered. These compounds have been called endocannabinoids. The most important are ara-chidonylethanolamide (anandamide), ara-chidonoylglycerol (2-AG), and 2- arachidonylglycerol ether **(Singh and Budhirja, 2006).** Enocannabinods are not stored in intracellular compartments, but are synthesized on demand by neurons and prephiral cells **(Basavarajappa, 2007)**.

Endocannabinoids have been demonstrated in many mammalian tissues and are widely distributed in the CNS, peripheral nerves, leukocytes, spleen and testicles **(Habyyeb et al., 2002)**. The uterus contains the highest levels of anandamide; the first discovered Endocannabinoid, suggesting a particular role of anandamide in female reproductive system (**Schmid *et al.*, 1997**).

Some investigators reported that cannabinoid receptors are expressed in the oviduct, uterus (**Paria et al., 1995**), and placental membranes (**Park et al., 2003**). Moreover, **Das et al. (1995)** demonstrated that $CB_1$ mRNA is present in mouse uterus and it showed a higher accumulation on days 4 and 7 of pregnancy than that on day 1**.** Moreover, **Fonesca et al. (2009)** found a

significant difference in expression of cannabinoid receptors during pregnancy being upregulated during mid-pregnancy, with decreasing density as gestation advances. These data indicate that enocannabinoids may play an important role during pregnancy and labor.

There are contradictory reports about the effect of endocannabinoides on uterine contractility. **Dennedy et al. (2004)** found a relaxant effect of the endocannbiniod, anandamide on uterine contractility and demonstrated that this relaxant effect may be linked to a reduction in uterine prostaglandin synthesis **(Dennedy et al., 2004).** In contrast, **Dmitrieva and Berkley (2002)** found an increase in the force of spontaneous uterine contraction under the effect of cannabinoid receptor agonists which was attributed, at least in part, to cannabinoid-induced production of PGE2 and PGF2α which decreases the intracellular concentration of cAMP **(Krall et al., 1984**).

As regards the mechanisms of action of endocannabinoids, many contradictory reports have been encountered. While some investigators reported that endocannabinoids directly regulate uterine contraction via binding with CB1 and CB2 receptors that preferentially couple to inhibitory $G\alpha_{i/o}$ proteins to inhibit adenylate cyclase activity, and hence reduce intracellular cAMP levels (**Pertwee et al., 1997; Mu et al 1999; Howlett and Mukhopadhyay, 2000**), others observed increased cAMP levels following CB1 activation (**Maneuf and Brotchie, 1997; Bonhaus et al., 1998; Busch et al., 2004**), implying possible coupling to $G\alpha_s$ proteins. Similar observations, however, were not reported for CB2 receptors (**Glass and Felder, 1997; Calandra et al., 1999**).

Numerous other signaling events, including increased activity of mitogen activated protein kinases (MAPKs) (**Bouaboula et al., 1995; Rueda et al., 2000**), inhibition of voltage-gated Ca2+ channels, activation of $K^+$ channels, and nitric oxide (NO) generation, have also been reported to follow CB receptor subtypes activation under different conditions (**Howlett et al., 2004; Demuth and Molleman, 2006**).

**Materials & Methods: 1-Animals:** Thirty healthy adult albino rats (24 female rats and 6 male rats) were obtained from the laboratory animals' farm unit Faculty of Veterinary Medicine, Zagazig University, with an average weight, 180-200 grams. The animals were kept in steel wire cages (6/cage) under hygienic conditions and kept on the diet which consisted of mixed commercial rat laboratory chow and supplied in separate clean containers. Animals had free access to water and kept at room temperature. All animals were bred in the animal house. The rats were accommodated to laboratory conditions for two weeks before the experiments going on. The male rats were used for induction of pregnancy.

***Groups:*** The animals were divided into four equal groups:

**Group (1):** consisted of six (6) adult non pregnant female rats to study the effects of the endocannabinoid, anandamide on spontaneous contractile activity of isolated uterine strips.

**Group (2):** consisted of six (6) pregnant female rats on day 10 of gestation to study the effects of anandamide on spontaneous contractile activity of isolated uterine strips.

**Group (3):** consisted of six (6) pregnant female rats on day 19 of gestation to study the effects of anandamide on spontaneous contractile activity of isolated uterine strips.

**Group (4):** consisted of six (6) pregnant female rats on day 10 of gestation to study the effects of anandamide on spontaneous contractile activity of isolated uterine strips in the presence of:

1. Selective cannabinoid CB (1) receptor antagonist, AM251.
2. Nitric oxide synthase inhibitor, $N^G$-nitro-L-arginine methyl ester (L- NAME).

3. Small conductance $Ca^{++}$ activated $K^+$ channels Blocker, Apamin.

**2-Drugs and chemicals:**

-Anandamide (Arachidonylethanolamide), non selective cannabinoid receptor agonist.

-AM251, selective cannabinoid CB (1) receptor antagonist.

1-$N^G$-nitro-L-arginine methyl ester (L-NAME), nitric oxide synthase inhibitor.

-Apamin, Blocker of small conductance $Ca^{++}$ activated $K^+$ channels.

*The previous chemicals were dissolved as follow*:

Anandamide was dissolved in ethanol.

AM251 was dissolved in dimethylsulfoxide (DMSO).

$N^G$-nitro-L-arginine methyl ester (L-NAME) and Apamin were dissolved in distilled water**.**

All the previous agents and their solvents were purchased from Sigma Chemicals CO. (Aldrich, St. Louis, Mo).

***** De Jalone solution: NaCl (18 gm/2 L),KCl (0.84 gm/2 L)**,**Glucose (2 gm/2 L), Na $HCO_3$ (2 gm/2 L), $CaCl_2$ (0.4 gm/2 L). The pH of this solution was 7.4 and it was bubbled with Carbogen (95%O2 and 5% CO2) to be used as a bath fluid for isolated uterine strips ***(Tong et al., 1995; Sharma et al., 1997)***.

All the chemicals used for preparing De-Jalone solution were purchased from El Nasr Pharmaceutical Chemicals CO. Abu Zaabal, Egypt.

**METHODS:**

**1- Preparation of the non pregnant group**

The non pregnant female rats were prepared with subcutaneous injection of estrogen (1 ml in sesame oil) for three successive days before the experiments for sensitization of the uterine smooth muscle.

**2- Timed- pregnant group:**

**Determination of the first day of pregnancy:**

Vaginal smears taken from the female rats were examined daily by using light microscope to ensure that they were in regular estrus cycle. The estrus phase of the estrus cycle was detected by the presence of cornified epithelial cells which increase in number and eventually predominate as the estrus progresses ***(Barcelona et al., 1977).*** The female proved to be in estrous phase was paired with a mature male rat in a separate cage. After mating, females were subsequently isolated until the time of analysis to ensure accurate gestation timing, and in the next morning a vaginal smear taken. Copulation was confirmed by the presence of a copulation plug or spermatozoa in the vagina. The presence of sperms indicated the first day of gestation **(*Klukovits et al., 2002).*** Parturition usually occurs in the evening of day 21 or the morning of day 22 as the duration of pregnancy in rats is about 21 days ***(Sladek and Robert, 1996).***

**3- Isolated uterine tissue protocol:**

Rats were sacrificed in the estrous phase in the non pregnant group (group 1), on day 10 of gestation (group 2 and 4), and on day 19 of gestation (group 3) by decapitation. The abdomen was opened, the uterine horns were dissected, and transferred immediately to a dish containing De-Jalone solution, then the extraneous tissues were removed e.g. pregnant uteri were cleaned from fat, placenta, fetus, fetal membrane and then rinsed thoroughly. Afterwards each horn was opened longitudinally along its mesenteric border and divided by a long cut into two equal length segments to produce strips of about 0.4 cm in width x 1.3 cm in length ***(Novaro et al., 1996).*** A thread was then attached to the end of each strip, and the preparation was mounted in De Jalone solution of pH 7.4 at temperature of 37°C, aerated with a mixture of 95% $O_2$ and 5% $CO_2$ in the organ bath which was relatively long and wide (50 ml volume) to prevent strip adhesion to the wall. One end of the strip was attached to a fixed pin in the aerator of the bath and the other to an ink writing lever. The load on the lever was 2-3 gm. The preparation required approximately 1

hour to equilibrate after dissection. The strips were bathed with De-Jalone solution. After spontaneous activity became regular various agents were added. After recording the effect of each dose, the uterine strips were washed 2 to 3 times with 5 minutes interval and left for about half an hour to return to their inherited conditions.

The drugs were added as follow:

***Anandamide*** was added in three separate doses: $10^{-6}$, $10^{-5}$ and $10^{-4}$ M/ml organ bath fluid (**Saitoh et al., 2007***)* to organ baths containing uterine strips isolated from;

a)-6 non pregnant adult female rats.

b)-6 rats on day 10 of gestation.

c)-6 rats on day 19 of gestation,

In additional experiments, the contractile activity of the uterine strips isolated from 6 rats on day 10 of gestation was recorded in response to addition of the third dose of ***anandamide*** ($10^{-4}$ M/ml) in the presence of:

-AM251 ($10^{-6}$ mol/ L) (**Saitoh et al., 2007***)*.

-$N^{G}$-nitro-L-arginine methyl ester (L-NAME) (3 x $10^{-5}$ mol/L) (**Yildirim et al., 2001***)*.

-Apamin ($10^{-8}$ mol/L) (**Modzelewska et al., 2003***)*.

The isolated uterine strips were incubated for 15 min with each of the previously mentioned chemicals followed by a period of 2-5 min incubation with *anandamide* ($10^{-4}$ M / ml). The amplitude (mm) and frequency (cycle/ 20min)Of contractions developed by the strips after the addition of each dose of anandamide alone or anandamide in the presence of different types of chemicals , were quantitated and expressed as the percentage of the amplitude or the frequency generated during the spontaneous contractile activity before the addition of these agents (the control).

**Statistical analysis:** All data were expressed as mean ± SE and statistically analyzed according to the methods described by ***Kirkwood (1989)*** using SPSS version 11.5. Differences were considered significant if <0.05.

## RESULTS

anandamide ($10^{-6}$ M/ml) produced a significant reduction in frequency (mean % of reduction 28.41±3.52) and amplitude (mean% of reduction 31.45±2.71) of spontaneous contractility of uterine strips isolated from pregnant rats on day 10 of gestation (P<0.001). In non pregnant and pregnant rats on day 19 of gestation anandamide ($10^{-6}$M/ml) produced insignificant (P>0.05) reduction of frequency (mean % of reduction 9.08±4.19; 3.5±2.21 respectively) and amplitude (mean % of reduction 2.23±1.29; 6.71±2.86).

Using anandamide in a dose of $10^{-5}$M/ml produced a significant reduction in frequency and amplitude of spontaneous contractility of uterine in all groups (P<0.001). The mean % of reduction of frequency was 42.8±2.82 in non pregnant; 67.3±3.19 in pregnant rats day 10 and 35.8±2.02 in pregnant rats day 19of gestation. The mean % of reduction of amplitude was 46.05±4.68 in non pregnant rats; 71.5±5.39 in pregnant rats day 10 and 32±4.02 in pregnant rats day 19 of gestation.

In dose $10^{-4}$ M/ml of anandamide there was a significant (P<0.001) reduction in frequency and amplitude of spontaneous contractility of uterine in non pregnant, pregnant rats on day 10 and pregnant rats on day 19 of gestation. The mean % of reduction of frequency was 70.7±6.6; 86.96±5.89 and 68.48±2.72 respectively. The mean % of reduction of amplitude was 74.73±5.87; 91.73±4.98 and 65.22±1.82 respectively.

The relaxant effect of anandamide in all doses ($10^{-6}$, $10^{-5}$, $10^{-4}$ M/ml) was significantly higher (P<0.001) in pregnant rats on day 10 of gestation compared with non pregnant rats and pregnant rats on day 19 of gestation.

Using Cb1 receptor antagonist AM251 significantly reduced the utero-relaxant

effect of anandamide (P<0.001) as addition of anandamide ($10^{-4}$M/ml) to uterine strips isolated from pregnant rats on day 10 of gestation pre-incubated with CB1 receptor antagonist AM251 ($10^{-6}$M/L) produced insignificant reduction (P>0.05) of frequency (mean% of reduction 5.6±2.52) and amplitude (mean% of reduction 9.22±5.31) of spontaneous contractility.

Incubation of uterine strips isolated from pregnant rats on day 10 of gestation with NO synthase inhibitor (L-NAME, $3x10^{-5}$M/L) significantly reduced (P<0.001) the effect of anandamide ($10^{-4}$M/ml) with mean % of reduction of frequency, 49.8±2.5 vs. 86.96±5.89 when anandamide ($10^{-4}$M/ml) was added alone and mean % of reduction of amplitude 53.53±3.49 vs. 91.73±4.98 when anan-damide ($10^{-4}$M/ml) was added alone.

Small conductance $Ca^{+2}$ activated $K^{+}$ channels blocker, Apamin significantly diminished the utero-relaxant effect of anandamide (P<0.001). Anandamide ($10^{-4}$M/ml) added to uterine strips isolated from pregnant rats on day 10 of gestation pre-incubated with Apamin ($10^{-8}$M/L) produced mean % of reduction of frequency 42.85±3.94 vs. 86.96±5.89 by using anandamide alone and reduction of amplitude 30.88±4.15 vs. 91.73±4.98 for anandamide alone.

**Fig (1):** representative recordings of the effect of anandamide (AEA $10^{-4}$M/ml) on spontaneous contractility of uterine strips isolated from non pregnant rats (a), pregnant rats on day 10(b) and pregnant rats on day 19 (c) of gestation.

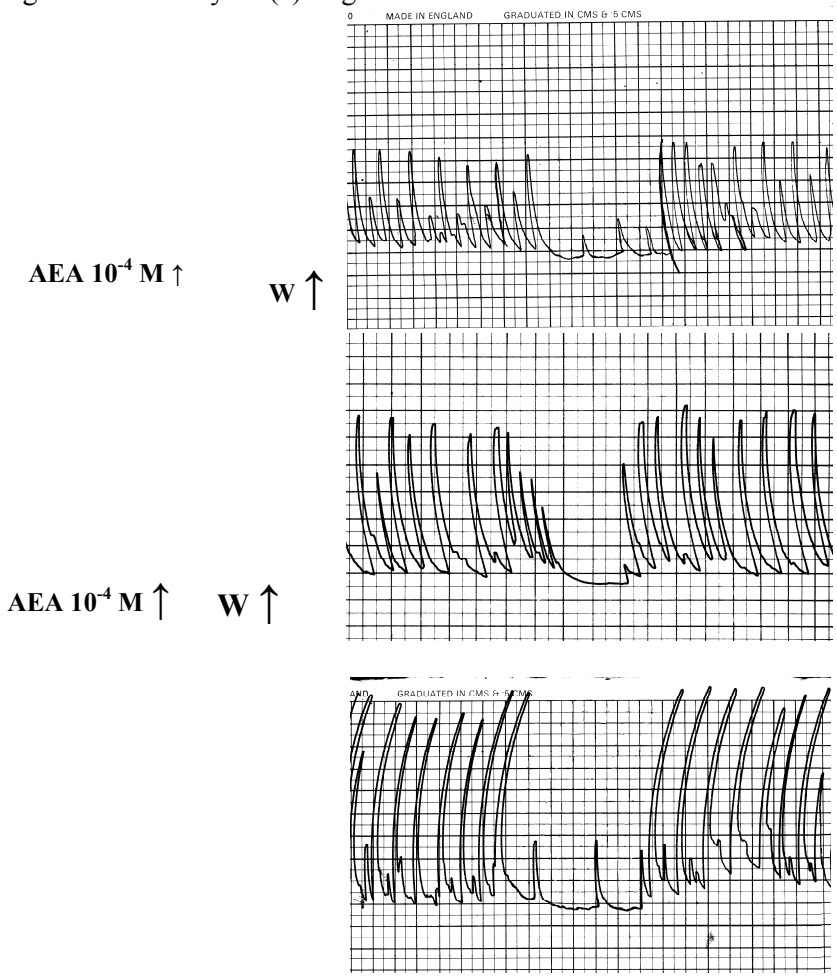


**Table (1):** Comparison between the percentages of reduction ( $\overline{X}$ **±SE)** of amplitude of spontaneous contraction of uterine strips isolated from non pregnant rats, rats on day 10 and day 19 of gestation in the presence of different doses of anandamide.

**Table (2):** Comparison between the percentages of reduction ( $\overline{X}$ **±SE)** of frequency of spontaneous contraction of uterine strips isolated from non pregnant rats, rats on day 10 and day 19 of gestation in the presence of different doses of anandamide.

| | **Percentage of reduction of amplitude** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Anandamide$10^{-6}$ M/ml | | | Anandamide$10^{-5}$ M/ml | | | Anandamide$10^{-4}$ M/ml | | |
| | Non pregnant | Day 10 | Day 19 | Non pregnant | Day 10 | Day 19 | Non pregnant | Day 10 | Day 19 |
| $\overline{X}$ | 2.23 | 31.4 | 6.71 | 46.05 | 71.5 | 32 | 74.73 | 91.73 | 65.22 |
| ±SE | 1.29 | 2.71 | 2.86 | 4.68 | 5.39 | 4.02 | 5.87 | 4.98 | 1.82 |
| F | 42.99***(P<0.001) | | | 17.924***(P<0.001) | | | 8.617**(P<0.01) | | |
| P of LSD | | <0.001 | N.S | | <0.01 | N.S | | <0.05 | N.S |
| | | | <0.001 | | | <0.001 | | | <0.01 |

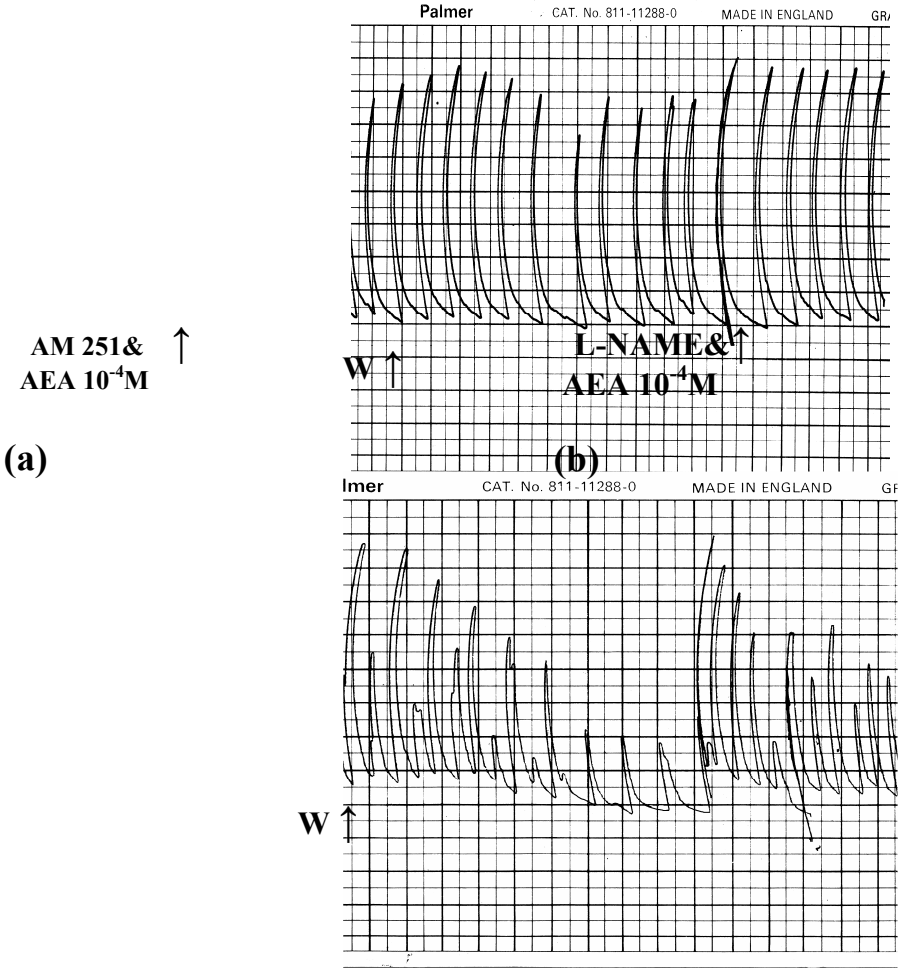
**Table (3):** Comparison between the percentages of reduction ( $\overline{X}$ **±SE)** of amplitude of contraction produced by addition of anandamide ($10^{-4}$ M/ml organ bath fluid) to uterine strips isolated from pregnant rats (day 10) before and after incubation with AM251 ($10^{-6}$ M/L), L-NAME (3 x $10^{-5}$ M/L), and Apamin ($10^{-8}$ M/L).

| | Percentage of reduction of amplitude | | | |
|---|---|---|---|---|
| | Anandamide ($10^{-4}$ M/ml) | AM251 & Anandamide ($10^{-4}$ M/ml) | L-NAME & Anandamide ($10^{-4}$ M/ml) | Apamin & Anandamide ($10^{-4}$ M/ml) |
| $\overline{X}$ | 91.73 | 9.22 | 53.53 | 30.88 |
| ±SE | 4.98 | 5.31 | 3.49 | 4.15 |
| F | 60.180***(P<0.001) | | | |
| P of LSD | | <0.001 | <0.001 | <0.001 |
| | | | <0.001 | <0.01 |
| | | | | <0.01 |

**Table (4):** Comparison between the percentages of reduction ( $\overline{X}$ **±SE)** of frequency of contraction produced by addition of anandamide ($10^{-4}$ M/ml organ bath fluid) to uterine strips isolated from pregnant rats (day 10) before and after incubation with AM251 ($10^{-6}$ M/L), L-NAME (3 x $10^{-5}$ M/L), and Apamin ($10^{-8}$ M/L).

| | Percentage of reduction of frequency | | | |
|---|---|---|---|---|
| | Anandamide ($10^{-4}$ M/ml) | AM251 & Anandamide ($10^{-4}$ M/ml) | L-NAME & Anandamide ($10^{-4}$ M/ml) | Apamin & Anandamide ($10^{-4}$ M/ml) |
| $\overline{X}$ | 86.96 | 5.60 | 49.845 | 42.85 |
| ±SE | 5.89 | 2.25 | 2.599 | 3.94 |
| F | 70.123***(P<0.001) | | | |
| P of LSD | | P<0.001 | P<0.001 | P<0.001 |
| | | | P<0.001 | P<0.001 |
| | | | | P>0.05 |

**Fig (2):** Representative recordings of the effect of anandamide (AEA $10^{-4}$M/ml) on spontaneous contractility of uterine strips isolated from pregnant rats on day 10 of gestation preincubated with AM251 $10^{-6}$ M/L (a) , L-NAME 3 x $10^{-5}$ M/L (b) and Apamin $10^{-8}$ M/L (c).




## DISCUSSION

Our results showed that anandamide exerted a significant dose dependant reduction in frequency and amplitude of spontaneous contraction of uterine strips isolated from both pregnant and non pregnant rats. This utero-relaxant effect of anandamide was almost completely abolished when anandamide was added after incubation of uterine strips isolated from pregnant rats on day 10 of gestation with the specific $CB_1$ receptor antagonist, AM251. This finding indicates that CB1 receptors are present in the rat uterus and may be the main receptor subtype involved in endocannabinoid-induced uterine relaxation.

These results are in accordance with those reported by **Dennedy et al. (2004)** who demonstrated that the endogenous cannabinoid, anandamide and the exogenous cannabinoid, $\Delta^9$-THC exerted a potent relaxant effect on human myometrial contractility. This relaxant effect was found to be prevented by CB1 antagonist SR141716 but not by CB2 antagonist SR144528.Therefore, they suggested that the relaxation component is under control of CB1 receptor only.

In support to the relaxant effect of anandamide on uterine smooth muscles that was observed in the present study, anandamide was found to have a CB1 mediated relaxant effect on vascular smooth muscles, leading to vasodilatation

and hypotension (**Zygmunt et al., 1997; Hillard, 2000**) and gastrointestinal smooth muscles, causing inhibition of gastrointestinal motility and gastric acid secretion (**Izzo et al., 2001**).

Our results are at variance with those of **Dmitrieva and Berkley (2002)** who found an increase in the force of spontaneous uterine contraction under the effect of cannabinoid receptor agonists.

In humans**,** while **Dennedy et al. (2004)** reported that the human endometrium expresses both CB1 and CB2 receptor subtypes, **Brighton et al. (2009)** found that $CB_1$ mRNA is expressed in the human myometrial smooth muscle cells and that $CB_2$ mRNA appears to be very low if present at all. In pregnant rats, **Buckly et al. (1998) and Fonesca et al. (2009)** described CB1 and CB2 receptor mRNA in the outer longitudinal and inner circular layer of the myometrium**.** In contrast, other investigators demonstrated that in mice, both CB1 and CB2 receptor subtypes are expressed in preimplantation embryos, whereas only CB1 is expressed in the oviduct and uterus (**Das et al., 1995; Paria et al., 1995, 2001; Wang et al., 2004**).

The first possible mechanism which accounts for the utero-relaxant effect of anandamide is that, under certain conditions, increased cAMP levels following CB1 activation have been observed (**Maneuf and Brotchie, 1997; Bonhaus et al., 1998; Busch et al., 2004**), implying possible coupling to $G\alpha_s$. Similar observations, however, were not reported for CB2 (**Glass and Felder,1997; Calandra et al., 1999**). Elevation of cAMP levels leads to smooth muscle relaxation because the increase in intracellular cAMP activates the cAMP-dependent protein kinase (PKA), which in turn phosphorylates the myosin light chain kinase and renders it inactive. This causes the myosin light chain to remain unphosphorylated and thus induces a relaxant response **(Lim et al., 2008)**. In contrast, others demonstrated that AEA signaling inhibits adenylate cyclase through binding with $G\alpha_i$ protein, thereby reducing cAMP levels (**Brighton et al., 2009**). However, an AEA-mediated reduction in cAMP levels does not result, as one may expect, in myometrial contraction (**Dennedy et al., 2004**), implying that alternative mechanisms control AEA-stimulated myometrial relaxation (**Brighton et al., 2009**). Furthermore, some investigators reported that smooth muscle relaxation could be mediated via either increase cAMP and this is probably due to increased intracellular binding of $Ca^{+2}$ ion, or decrease cAMP which is associated with increased $Ca^{+2}$ ion efflux from the muscle cells (**Ganong, 2009**).

The second possible mechanism explaining the utro-relaxant effect of anandamide is the reduction of intracellular $Ca^{+2}$ concentrations. CB1 receptor signaling is known to inhibit L-type $Ca^{2+}$ channels and inhibit intracellular $Ca^{2+}$ store release in muscle cells leading to relaxation (**Gebremedhin et al., 1999; Hogestatt and Zygmunt, 2002**). It has been reported that only $CB_1$ and not CB2 regulates ionic currents (inhibition of voltage-gated L, N and P/Q-type $Ca^{2+}$ channels, activation of $K^+$ channels) **(Howlett *et al.*, 2004; Demuth and Molleman, 2006).**

**Noble et al.** (**2010**) showed that all small conductance $Ca^{2+}$ activated $K^+$ (SK) channel isoforms (SK1–3) are expressed and translated throughout pregnancy in pregnant rat myometrium and they contribute more to quiescence than large conductance $Ca^{2+}$-activated $K^+$ (BK) channels. Due to a constitutive association with calmodulin, SK3 channels are highly sensitive to changes in cytosolic $Ca^{2+}$ levels (**Bond et al., 1999; Xia et al., 1998**) and are thus capable of exerting abrupt negative feedback regulation of intracellular $Ca^{2+}$ (**Brown et al., 2007**).

The present study revealed that, the relaxant effect of anandamide on

spontaneous uterine contraction was partially prevented by incubation of uterine strips with Apamin which is a small conductance-$Ca^{+2}$ activated $K^+$ channel blocker. Therefore the third possible mechanism which explains the utero-relaxant effect of anandamide is the activation of $K^+$ channels. These results are in agreement with those of many investigators who demonstrated that activation of $K^+$ channels is one of the signal-transduction pathways regulated by CB1 receptor (**Howlett *et al.*, 2004; Demuth and Molleman, 2006**). Furthermore, **Baldassano et al. (2007)** showed that the in vitro spontaneous mechanical activity of longitudinal smooth muscle in mouse ileum was reduced by anandamide in a concentration-dependent manner and observed that this reduction was almost abolished by Apamin.

Our results also showed that the relaxant effect induced by anadamide was partially blocked by incubation of uterine strips isolated on day 10 of gestation with NO synthase inhibitor, L-NAME. This finding indicates that, the fourth possible explanation of the utero-relaxant effect of anandamide is the generation of NO.

Our results are supported by the findings of **Maccarrone *et al.* (2000)** who reported that activation of CB1 cannabinoid receptors by AEA causes a stimulation of the inducible NO synthase activity. Also it was found that methanandamide, the stable synthetic analogue of anandamide, induced iNOS protein expression and NO production in uterine explant tissue (**Vercilli et al., 2009**).

Moreover, throughout gestation myometrial NO production is up regulated to reach high levels in midgestation despite low circulating level of anandamide at this time of pregnancy. This up regulation of NO production could be attributed to the up regulation of cannabinod receptors in midgestation and thus may contribute to pregnancy maintainance by inhibiting uterine smooth muscle contraction (**Izumi et al., 1993; Riemre et al., 1997; Suzuki et al., 2009**). Then, close to term NO production decreases in the myometrium thus promoting effective contractions that result in labour (**Maul et al., 2003; Suzuki et al., 2009**). In contrast to the myometrium, NO production in the cervix is low during gestation and becomes upregulated once pregnancy advances to term thus helping cervical dilatation during labour (**Maccarrone *et al.*, 2008**).

In addition, it was reported that CB1 activates, whereas CB2 inhibits nitric oxide synthase (**Howlett et al., 2004; Demuth and Molleman, 2006**). The opposite effect of CB1 versus CB2 on nitric oxide (NO) release might be relevant for the in vivo control of reproduction. Since human endometrium expresses both CB1 and CB2 (**Dennedy et al., 2004**), it is believed that these two receptor subtypes are engaged at different time points to modulate in opposite ways NO content and thus NO-dependent effects (**Maccarrone *et al.*, 2008**).

Another possible mechanism of action is that anandamide may be related to alteration in myometrial gene expression. **Brighton et al. (2009)** demonstrated that AEA activates ERK1/2 in human myometrial cells. ERK1/2 proteins are members of the MAPK family, which can provide a link between extracellular stimuli and transcription factors to regulate gene expression. These effects were mediated directly through CB receptor-$G\alpha_{i/o}$ coupling. Indeed, longer term AEA exposure suppresses calponin and smoothlin expression in ULTR cells. Taken together these data suggest that AEA may further confer a relaxatory phenotype on the myometrial cells (**Tylor et al., 2007**).

One of the outstanding observations in the present study is that the utero-relaxant effect of anandamide was more potent in uterine strips isolated from pregnant rats on day 10 of gestation than that in both non pregnant rats and pregnant rats on day 19

of gestation. This can be explained by the results of **Fonesca et al. (2009)** who found a significant difference in the protein levels of cannabinoid receptors between days of gestation in rat uterus. CB1 protein levels on day 10 and 12 were significantly higher than those on days 16 and 19 of pregnancy. They also detected immunoreactivity for CB1 receptors in the circular muscle layer that was upregulated during midpregnancy, with decreasing intensity as gestation advances. In addition, it was found that $CB_1$ mRNA is present in mouse uterus and it shows a higher accumulation on days 4 and 7 of pregnancy than that on day 1**(Das et al., 1995)**.

Anandamide has also been shown to have non CB1 and non CB2-dependent effects suggesting the existence of a CB3 receptor **(Fride et al., 2003)** and evidence exists that anandamide can also bind to other receptors that are not exclusively associated with cannabinoids **(Brown, 2007)**. It has been shown that anandamide binds to and activates the transient receptor potential vanilloid 1 receptor (TRPV1 or VR1), which is characterized as a ligand-gated non selective cationic channel (**Caterina et al., 1997; Stelt et l., 2004**). The concentrations of AEA required to fully activate TRPV1 as assessed by measuring intracellular $Ca^{+2}$ are 1- to 10-fold higher than those required to evoke CB1-mediated functional responses (**Zygmunt et al., 1999; De Petrocellis et al., 2000; Smart et al., 2000; Ross et al., 2001**). A strong reactivity for the vanilloid receptor in the longitudinal muscle layer of rat uterus was detected throughout gestation **(Fonesca et al., 2009)**. In human, a dramatic increase in plasma anandamide levels during term labour compared with non-labouring women has been described suggesting a role for anandamide in labour (**Habayeb et al., 2004**). Thus, it was hypothesized that TRPV1 activation mediated by the high levels of anandamide might contribute to the ability of the outer myometrial layer to generate optimal contractile activity during labour **(Fonesca et al., 2009)**.

There are some limitations in the extrapolation from in vitro studies to the in vivo situation and from animal to human studies. The in vitro studies do not account for a possible central effect of endogenous cannabinoids which may have further relaxant effects on peripheral smooth muscle tissues **(Ameri, 1999).** Further studies are required to evaluate the cannabinoid effects on human uterine tissue during pregnancy, in comparison with nonpregnant myometrium and to examine the possible mechanisms of this effect. Further studies are also needed to investigate the effects of cannabinoids on the fetus or the feto-placental circulation.

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## التغيرات المحدثة بالكانبينويد في انقباضية رحم الفئران

أكتشفت مركبات الإندوكنابينويد في كثير من الخلايا و تنتشر بشكل واسع في الجهاز العصبي المركزي، الأعصاب الطرفية ، كرات الدم البيضاء ، الطحال و الخصية.
وقد سجل العلماء ان التعرض للكنابينويد الخارجية كالماريجوانا يصاحبه تأثيرات سيئة علي الحمل مثل الولادة المبكرة، تأخر نمو الجنين و فشل الحمل. كما وجد أيضا أن مستقبلات الكنابينويد موجودة في قناة المبيض، الرحم و أغشية المشيمة. وكذلك يحتوي الرحم علي أعلي نسبة من مركب الأنانداميد وهو أول إندوكنابينويد تم أكتشافه مما يرجح بأن الأنانداميد يلعب دورا في عملية الإنجاب.

و بسبب وجود تقارير متضاربة حول تاثير مركبات الكنابينويد علي انقباضية الرحم و تقارير متضاربة عن كيفية هذا التاثير لذلك صممت هذه الدراسة لبحث تأثير الأنانداميد علي الانقباضية التلقائية لرحم الجرذان الحوامل و غير الحوامل. ولإيضاح الكيفية التي يؤثر بها الأنانداميد علي الرحم و مدي ارتباطها بمستقبلات الكنابينويد (١)، اكسيد النيتريك و ممرات البوتاسيوم التي تنشط بالكالسيوم.

أجريت هذه الدراسة علي عدد كلي٣٠من الجرذان البيضاء البالغة (٢٤إناث و٦ ذكور) و استخدمت الذكور لاحداث الحمل. و تم تحديد اليوم الول من الحمل بوجود حيوانات منوية في الفحص الميكرسكوبي لمسحة من الافرازات المهبلية. و قد قسمت الاناث إلي أربع مجموعات متساوية تحتوي كل منها علي ٦ فئران. ثلاث من هذه المجموعات (غير الحوامل ، حوامل في اليوم العاشرمن الحمل، و حوامل في اليوم التاسع عشرمن الحمل) استخدمت لدراسة تأثيرالأنانداميد علي الانقباضية التلقائية لشرائح رحم الجرذان المعزولة. والمجموعة الرابعة (حوامل في اليوم العاشرمن الحمل ) استخدمت لدراسة الكيفية التي يؤثر بها الأنانداميد علي الرحم وذلك باستخدام مضاد مستقبلات الكنابينويد (AM251) ، مثبط انزيمات تصنيع اكسيد النيتريك ( L-NAME) ، و غالق ممرات البوتاسيوم التي تنشط بالكالسيوم (Apamin).

و قد أظهرت الدراسة أن الأنانداميد له تأثير إنبساطي ذو دلالة احصائية علي شرائح الرحم المعزولة من الجرذان الحوامل وغير الحوامل و يزيد هذا التأثيربزيادة تركيز الأنانداميد. و أن هذا التأثيرالانبساطي للانانداميد يحدث غالبا عن طريق التاثير المباشر علي مستقبلات الكنابينويد (١) لأن مضادات هذه المستقبلات ألغت هذا التأثير بشكل شبه كامل مما يوحي بان مستقبلات الكنابينويد (١) موجودة في رحم الجرذان و انها ربما تكون النوع الرئيسي من مستقبلات الكنابينويد المسؤل عن هذا التاثير الانبساطي للانانداميد. و قد أظهرت الدراسة ايضا أن كل من مثبط انزيمات تصنيع اكسيد النيتريك، و غالق ممرات البوتاسيوم التي تنشط بالكالسيوم تقلل بشكل ذو دلالة احصائية التأثير الإنبساطي للأنانداميد.

كما أظهرت الدراسة أن التأثيرالإنبساطي للأنانداميد كان اقوي بدرجة ذات دلالة احصائية علي شرائح الرحم المعزولة من الجرذان الحوامل في اليوم العاشرمن الحمل مقارنة بكل من مجموعة الحوامل في اليوم التاسع عشرمن الحمل وكذلك غير الحوامل.

و مما سبق يمكن ان نستنتج أن:

- الأنانداميد له تأثير إنبساطي علي الانقباضية التلقائية لرحم الجرذان الحوامل و غير الحوامل و أن هذا التأثير اقوي في منتصف الحمل مما قد يساعد علي هدوء الرحم خلال فترة الحمل ثم ينخفض هذا التأثير الإنبساطي في نهاية الحمل مما قد يساعد علي انقباض فعال للرحم اثناء الولادة.
- الأنانداميد يحدث هذا التأثير الإنبساطي المباشر غالبا عن طريق ارتباطه بمستقبلات الكنابينويد (١). كما أن كل من زيادة انتاج اكسيد النيتريك و فتح ممرات البوتاسيوم التي تنشط بالكالسيوم يلعب دورا هاما في هذا التأثير الإنبساطي.
- تلقي هذه الدراسة الضوء علي احتمالية وجود دورا فسيولوجيا تلعبه مركبات الاندوكنابينويد اثناء فترة الحمل و الولادة. كما تدعم الراي القائل بان استخدام مركبات الكنابينويد الخارجية له تاثير سلبي علي الحمل و الولادة.