Non-genomic uterorelaxant actions of corticosteroid hormones in rats: An in vitro and in vivo study

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ABSTRACT

Aims: We aimed to identify the short-term effects of a glucocorticoid (GC) and a mineralocorticoid (MC) on non-pregnant and late pregnant rat uterine contractions to estimate their tocolytic potential.

Methods: The in vitro contractility studies were performed with uterine tissues from non-pregnant and 22-day pregnant SPRD rats. The cumulative dose-response of fludrocortisone (FLU) and dexamethasone (DEX) was measured alone or in the presence of steroid receptor antagonist mifepristone (MIF) or spironolactone (SPR). [^{35}S] GTPγS and cAMP immunoassays were carried out to detect the activated G-proteins and cAMP, respectively. The in vivo uterine action of single doses of FLU and DEX was measured by smooth muscle electromyography. The results were statistically analyzed with an unpaired *t*-test.

Results: FLU and DEX relaxed both pregnant (33 and 34%) and non-pregnant (37 and 34%) uteri in vitro. MIF inhibited the relaxing effect of DEX, especially in the pregnant uterus, but reduced the effect of FLD only in non-pregnant tissues. GTPγS studies showed a MIF-sensitive elevation in activated G-proteins both in pregnant and non-pregnant uteri by DEX, whereas FLU induced activation only in non-pregnant samples. DEX relaxed pregnant and non-pregnant uteri in vivo in a MIF-sensitive way.

Significance: DEX can inhibit contractions in the late pregnant uterus in a non-genomic manner, while FLU seems to be ineffective. Its action is mediated by a G-protein-coupled receptor that can be blocked by mifepristone. Further investigations are necessary to determine the required dose and duration of GCs in the therapy of premature birth.

1. Introduction

Steroid hormones exert their physiological response on targets by mediating the genomic pathway, which is known as the classical steroid mechanism. Besides, there is the non-genomic mechanism of action, which is usually a prompt response, such as signaling cascade modification, and defines those pathways that have no direct influence on gene expression (Lösel and Wehling, 2003).

The fast action of glucocorticoids (GCs) was described proving that the administration of GC led to the fast inhibition of stimulus-induced adrenocorticotropic hormone (ACTH) secretion (Dallman, 2005). Corticosterone inhibits the hypothalamic–pituitary–adrenocortical axis in a fast manner, and stimulates an adaptive reflex to the situation, such as cognitive aspects (Groeneweg et al., 2012). Furthermore, it can also temporarily elevate the levels of excitatory amino acids aspartate and glutamate (Falkenstein et al., 2000). Corticosterone quickly elevates blood sugar during stress by inhibiting glycogenesis in high concentrations in the early stages of stress response (Jiang et al., 2014), therefore, the GC fast action also increases carbohydrate ingestion (Dallman, 2005) and decreases insulin secretion (Tasker et al., 2006). Besides, a beneficial fast effect of GC has been reported on airway smooth muscles in asthmatic condition (Panettieri et al., 2019). In addition, GCs mediate a T-cell immunosuppressive response by non-genomic action (Alangari, 2010). The uterine contraction of non-pregnant rats can be inhibited by cortisol (Gutiérrez et al., 1994; Perusquía, 2001) and dexamethasone (Henry et al., 1973). Endometrial GS deficiency increases inflammation and angiogenesis, and therefore leads to heavier menstrual bleeding due to the impaired subsequent vasoconstriction (Critchley et al., 2020).
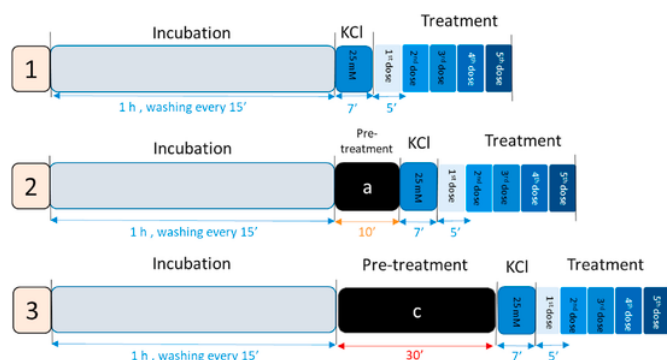
Fig. 1. Protocol for the in vitro contractility study. The incubation period was 1 h with washing the chambers every 15 min. KCl stimulation was for 7 min, and then minimum 5 doses of each drug were given for 5-min intervals in a cumulative manner; (1) denuded myometria or intact uterine samples of pregnant and non-pregnant animals with dexamethasone (DEX) and fludrocortisone (FLD); (2) samples with pre-treatment (10 min) with steroid receptor antagonist (a): spironolactone 10^{-6} M for FLD, mifepristone 10^{-8} M for DEX and 10^{-6} M for both steroids, then treatment with DEX and FLD; (3) sample with actinomycin D10^{-6} M pre-treatment (30 min) then treated with DEX or FLD.
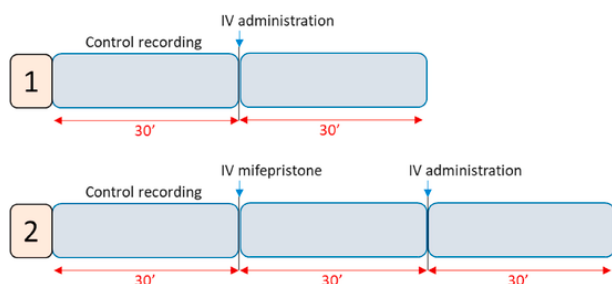

Fig. 2. Protocol for the in vivo contractility study. The animals were first anesthetized, and strain gauges or electrodes were inserted on the abdomen of non-pregnant or pregnant animals, respectively. The 30-min recording of spontaneous contractions (control period) was the same for all experiments; (1) dexamethasone (DEX) (4 mg/kg) or fludrocortisone (FLD) (25 mg/kg) was administered IV after the control period. (2) Following the control period, mifepristone (MIF) in 10 mg/kg was injected IV, then DEX or FLD was administered IV 30 min after MIF administration.

The fast response of aldosterone (ALD) on the cardiovascular system was reported after 5 min of its administration, when peripheral vascular resistance and cardiac output were increased and decreased, respectively. Due to the short timeframe of response, the hypothesis of a new non-genomic pathway was made (Schmidt, 2008). ALD has been reported to change the cell volume of human mononuclear leukocytes by modifying the Na^+/H^+ antiporter and inducing alkalization. This action was not blocked by mineralocorticoid receptor (MR) blocker spironolactone (SPR) within15 min (Lösel and Wehling, 2003). In addition, ALD has a rapid positive inotropic effect on rat cardiac muscle (Barbato et al., 2002), and its fast effect has been reported on $Na^+/K^+/2Cl^-$ cotransporter and Na^+/K^+ pump activity in rabbit cardio-myocytes (Mihailidou and Funder, 2005). In the collecting ducts, ALD induces Na^+/H^+ exchange and promotes intercellular Ca^{2+} flux, but in the medullary thick ascending limb, it decreases Na^+/H^+ exchange and bicarbonate absorption in a non-genomic way (Hammes and Levin, 2007). ALD can also moderately inhibit the uterine contraction in Wistar rats (Gutiérrez et al., 1994).

In our previous work, we reported the non-genomic actions of sex steroids on pregnant uterine smooth muscles in rats (Mirdamadi et al., 2020). In the present study, we aimed to investigate the fast, non-genomic action of MC fludrocortisone (FLD) and GC dexamethasone (DEX) on uterine contractions and signaling pathways both in pregnant and non-pregnant rats.

2. Materials and methods

2.1. Animals

Sprague-Dawley rats (180–200 g, Animalab Hungary Ltd, Vác, Hungary) were kept under controlled temperature, humidity, and light (20–23 °C and 40–60% and 12 h light/dark regime, respectively). Diet (Altromin 1324, Charles-River Laboratories, Sulzfeld, Germany), and tap water were available ad libitum. The animals were treated in accordance with the European Communities Council Directive (2010/63/EU) and the Hungarian Act for the Protection of Animals in Research (Article 32 of Act XXVIII). All experiments involving animal subjects were carried out with the approval of the National Scientific Ethical Committee on Animal Experimentation (registration number: IV./3071/2016.).

For the experiment, we used non-pregnant rats in the estrus phase and 22-day pregnant rats. The estrus phase was detected by vaginal impedance with Estrus Cycle Monitor (IM-01, MSB-MET Ltd., Balatonfüred, Hungary). For mating, rats in the estrus cycle were chosen and placed separately in an automated breeding cage with male rats (240–260g). The sexual intercourse was evaluated by native vaginal smear or copulation plugs. The confirmed pregnant animals were kept in new cages. The positive cases were considered as first-day pregnant animals.

2.2. Isolated organ bath contractility studies

The uterine samples from animals terminated in a carbon dioxide chamber were collected, cleaned, and cut to 3–4 mm dissections. The samples were collected from 2 animals and both sides of the uterine horn were used in a single experiment (8 rings/experiment) repeated at least 3 times. The uterine rings were tied with silk thread and vertically mounted in isolated organ baths which were filled with 10 ml of de Jongh buffer made of 137 mM (mM) NaCl, 3 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 12 mM NaHCO₃, 4 mM NaH₂PO₄, 6 mM glucose, the pH and the temperature were adjusted to 7.35–7.40 and 37 °C, respectively. The chambers were continuously bubbled through with carbogen (95% O₂ + 5% CO₂). The samples were fixed from the bottom to the tissue holder and the top of the tissues was attached to a gauge transducer (SG-02; MDE Gmbh., Heidelberg, Germany), with a primary resting tension of 1.5 g, the sensors from the gauge measured the contractions. The data were recorded and analyzed with a SPEL Advanced ISOSYS Data Acquisition System (MDE Gmbh., Heidelberg, Germany). The equilibrium period was 1 h, and during this period the tissues were washed every 15 min.

Rhythmic contraction was achieved by adding 25 mM KCl to each chamber for 7 min. Steroids were added separately in a cumulative dose manner: dexamethasone (DEX, 10^{-6}- 10^{-4} M) and fludrocortisone (FLD, 10^{-8}-10^{-4} M) for 5 min for each dose. The concentration-response curves were plotted, and the results were expressed in percentage change as compared to the initial KCl-induced contraction.

To investigate the role of classical steroid receptors, the tissues were pre-treated with the following receptor antagonists for 10 min before KCl stimulation: spironolactone in10^{-6} M for FLD (Mihailidou and Funder, 2005), mifepristone (MIF) in 10^{-8} M for GR (Attardi et al., 2004), and in 10^{-6} M (Mirdamadi et al., 2020) for both steroids mentioned. In another set of experiments, uterine tissues were pre-treated with the transcription inhibitor actinomycin D (10^{-6} M) (Pérez et al., 2013), for 30 min before adding DEX or FLD.

In order to check the possible role of endometrium in the fast action of steroids, the endometrium of the uterine tissue was removed by scratching, and the experiments were carried out on the denuded myometrium as described above for the whole uterus (Fig. 1).
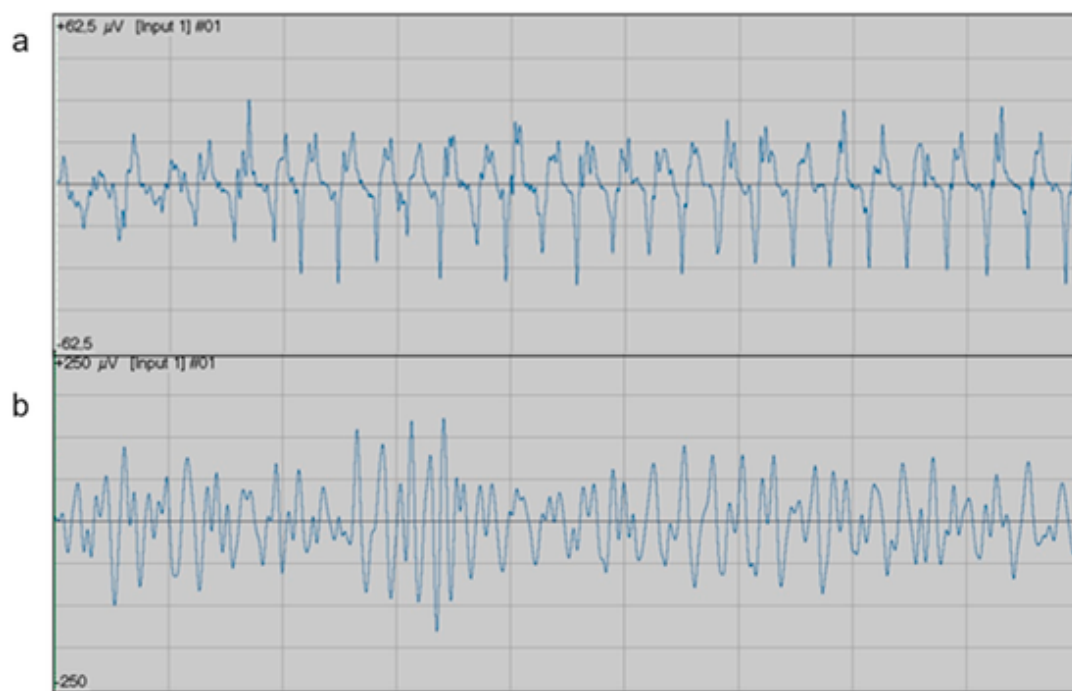
Fig. 3. Representative primary signals in rat uterus within 30 min of experiment. a: mechanical signals of the non-pregnant tissue. The mechanical contractions were measured with strain gauges sutured onto the surface of the uterus. AUC analysis was then performed on the recorded mechanical signals. b: myoelectric signals of the 22-day pregnant tissue. The myoelectric signals of the myometrium were detected with silver disk electrodes positioned on the abdomen. The recorded myoelectric signals were then analyzed by fast Fourier transformation (Szucs et al., 2017).

2.3. In vivo studies

Non-pregnant (Groups 1, 2, and 3), and 22-day pregnant (Groups 4, 5, and 6) rats were anesthetized with isoflurane inhalation and the jugular vein was cannulated for later intravenous drug administration.

In the case of the non-pregnant experiment after laparotomy, an implantable strain gauge was sutured onto the surface of the left uterine horn, while for the pregnant study a bipolar disk electrode pair was fixed subcutaneously 1 cm right from the midline above the uterus. To cover the incision, the surfaces of the abdominal wall were closed with surgical staples. Both the mechanical (strain gauges) and myoelectric signals (disk electrode) were recorded for 30-min time intervals both before and after the administration of the investigated drugs (Fig. 2).

Rats in Group 1 ($n = 6$) and Group 4 ($n = 7$) were treated with DEX (4 mg/kg IV) (Narayanan, 1983) only, while animals in Group 2 ($n = 6$) and Group 5 ($n = 7$) received MIF (10 mg/kg IV) (Curry and Nothnick, 1996) and then DEX (4 mg/kg IV) 30 min apart. Group 3 ($n = 4$) and Group 6 ($n = 6$) were treated with FLD (25 mg/kg IV) (Laviolle et al., 2014). In non-pregnant rats, the uterine contractions were evaluated by the AUCs of the recorded contraction. In pregnant rats, the electromyographic responses were evaluated by fast Fourier transformation (FFT) and the maximum of power spectrum density (PsD_{max}) values were compared in the frequency range of 1–3 cpm, which is characteristic for late pregnant uteri (Fig. 3) (Szucs et al., 2017).

2.4. [^{35}S]GTPγS studies

To investigate the efficacy of steroids on G-protein-coupled receptors (GPCRs), especially $G_{\alpha i/o}$ in a non-genomic manner, the changes of GDP to GTP were measured with radiolabeled, non-hydrolysable GTP. The experiment of [^{35}S]GTPγS was performed as described in our previous study (Mirdamadi et al., 2020). Uterine tissue samples of both pregnant and non-pregnant animals ($n = 10$) were grounded and homogenized with Ultra-Turrax® (IKA-Werke GmbH & Co. KG, Staufen in Breisgau, Germany) in an ice bath for 2×30 s with 20 vol (W/V) of ice-cold Tris-EDTA buffer (composed of 10 mm Tris–HCl, 1 mM EDTA, 0.6 mM MgCl$_2$, and 0.25 M sucrose, pH 7.4), and afterwards suspended by a 4-layer gauze filter. Then the pellets were suspended by centrifugation at 40,000g for 20 min at 4 °C. Finally, the protein content of the pellets was measured with a Nanodrop^{TM} 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, US) and the pellets were diluted to 10 mg/ml sample.

The uterine tissue fractions (in a final concentration of 10 μg/ml) and Tris-EGTA buffer (pH 7.4) composed of 50 mM Tris-HC, 1 mM EGTA, 3 mM MgCl$_2$, 100 mM NaCl, containing 20 mbq/0.05 cm^3 [^{35}S]GTPγS (0.05 nm) were pre-incubated in 24 polystyrene test tubes (Starstedt Co.) with or without MIF (10^{-6} M) at 30 °C. After 15 min, different doses of DEX (10^{-6}-10^{-4}) and FLD (10^{-8}-10^{-4}) were added separately for 20 min. Total binding or basal activity and non-specific binding were evaluated by measuring the buffer without protein sample as basal activity and 10 μm unlabeled GTPγS and subtraction from total binding for non-specific binding. The reaction in the incubation period was terminated by fast vacuum filtration (through Whatman GF/B filters with Brandel M24R Cell harvester). To separate the bound and free [^{35}S]GTPγS completely, the filters were washed with ice-cold buffer (pH 7.4) 3 times. Then the filters were dried, and their radioactivity was detected in Ultima Gold^{TM} MV aqueous scintillation cocktail with Packard TriCarb 2300 TR liquid scintillation counter. Each experiment was designed in triplicate, repeated 2 times.

2.5. Cyclic AMP studies

The changes in cyclic AMP (cAMP) levels of the sample tissue by non-genomic action of steroids were investigated using the commercial cAMP Enzyme Immunoassay Kit (Cayman Chemical, USA). The experiment was followed by an isolated organ bath study. Uterine tissues of both pregnant and non-pregnant SPRD rats were collected and incubated in an organ bath filled with 10 ml of de Jongh buffer. After incubation with MIF (10^{-6} M) (half of the samples - the other half without
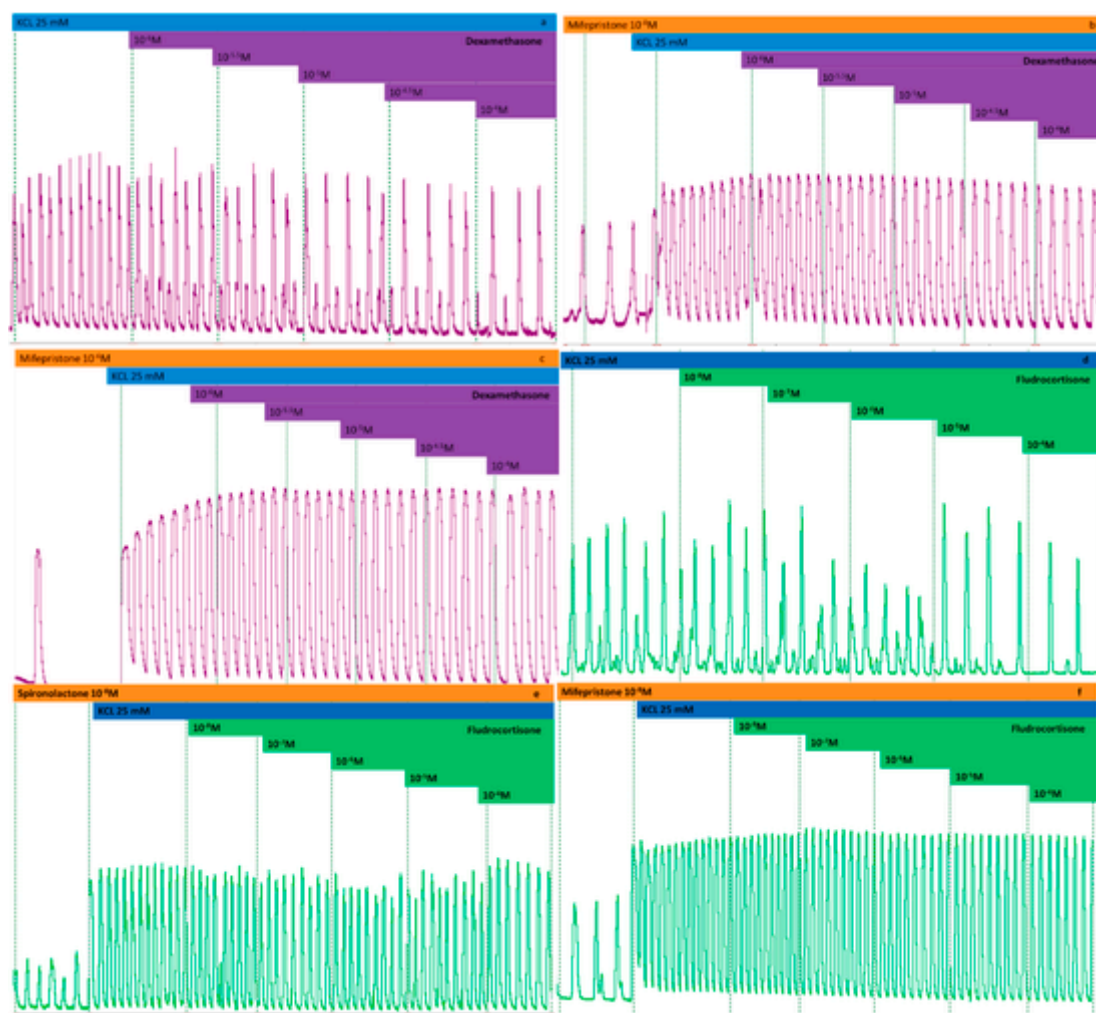
Fig. 4. Changes in KCL induced (25 mM) non-pregnant uterine contraction by dexamethasone (10^{-6} -10^{-4} M) (a) and fludrocortisone (10^{-8}- 10^{-4} M) (d). the effects were investigated in presence of mifepristone 10^{-8} M for dexamethasone(b), spironolactone 10^{-6} M for fludrocortisone (e) and mifepristone 10^{-6} M for both drugs (c and f). Each figure is a representative record.

mifepristone) for 10 min and KCl 25 mM for 7 min, 2 doses of DEX (10^{-4}-10^{-5} M) and FLD or control (10^{-4}-10^{-6}) were added for 5 min. Finally, forskolin (10^{-5} M) was added to all chambers for another 10 min. Then, by using liquid nitrogen, the samples were immediately frozen and kept at −70 °C. Liquid samples were prepared from frozen tissues. After weighing, the frozen tissues were pulverized, homogenized, mixed with 10 vol of an ice-cold aqueous solution of 5% trichloroacetic acid (TCA), and centrifuged at 1500 g for 15 min. The supernatants were separated from TCA with water-saturated ether. The separation was repeated 3 times. Ether residue was evaporated by heating the liquids and the final liquid samples were stored at −70 °C to be used in the cAMP assay. The cAMP level of the samples was expressed in nmol/mg tissue.

2.6. Drugs and chemicals

Dexamethasone sodium phosphate (dexamethasone 21-phosphate disodium - DEX) was purchased from TEVA Pharmaceutical, Budapest, Hungary. Mifepristone (MIF) fludrocortisone (FLD) and spironolactone (SPR) were all purchased from Sigma-Aldrich, Budapest, Hungary. Forskolin was purchased from Tocris, Norderstedt, Germany.

DEX was dissolved in distilled water for both in vivo and in vitro experiments. For the in vitro study, FLD was dissolved in water; ethanol; Macrogol 400 = 90; 0.2; 0.8 in the highest concentration, further dilutions were made with water. In the in vivo experiments, FLD was dis-

solved in water; dimethyl sulfoxide (DMSO); Macrogol 400 = 12.5; 12.5; 75.

2.7. Statistical analysis

The response curves for the in vitro experiments were plotted by analysis of the area under the curve (AUC) of contraction response against concentration. Based on the evaluated AUCs, the E_{max} and EC_{50} values were determined and presented as the mean ± SEM.

The recorded mechanical and myoelectric signals of the in vivo experiments were analyzed by AUC and fast Fourier transformation, respectively. The AUC and PsD_{max} values were determined and compared statistically (Szucs et al., 2016, 2017).

Data analysis and statistical assays were done by using the Prism 8.0 (GraphPad Software Inc. San Diego, CA, USA) computer program by applying the ANOVA Dunnett's test.

3. Results

3.1. Non-pregnant uterus

3.1.1. Isolated organ bath studies

DEX elicited a moderate relaxing effect (37%) at high concentration (10^{-4} M) (Fig. 4..a, Fig. 5a), and FLD showed a similarly weak relaxing action (34%) at the highest concentration (10^{-4} M) (Fig. 4 d, Fig. 5b).
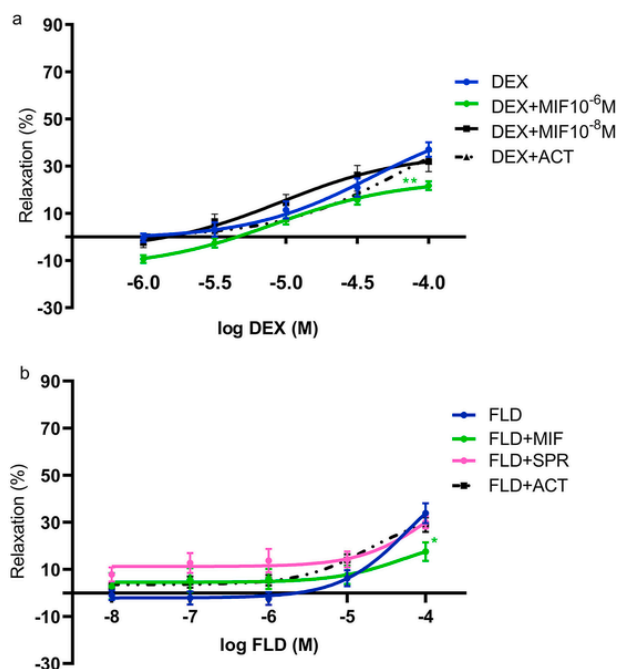
Fig. 5. Effects of dexamethasone(a) and fludrocortisone(b) on non-pregnant uterine contractions in vitro stimulated with KCl (25 mM) and pre-treated with spironolactone(10^{-6} M) for fludrocortisone or mifepristone (10^{-8} M) for dexamethasone and mifepristone (10^{-6} M) for non-genomic steroid action, Actinomycin D for both drugs separately, presented as a percentage of relaxation. *: $p < 0.05$; **: $p < 0.01$; ACT, Actinomycin D; DEX, dexamethasone; FLD, fludrocortisone; MIF, mifepristone.

The relaxing effects of both drugs at the concentration of 10^{-4} M were inhibited by the higher dose of MIF (10^{-6} M), while nor lower dose of MIF neither SPR were changed significantly the response of DEX and SPR (Fig. 4b, c, e, and f, Fig. 5a and b). Actinomycin D pre-treatment did not modify the effect in either DEX or FLD (Fig. 5a and b). Endometrium removal did not change the action either (data not shown). In the subsequent experiments for the investigation of the signaling pathway, the action of DEX alone and in the presence of MIF was measured. Since there were no significant changes with the intervention (endothelium removal) and the lower dose of MIF (10^{-8} M), we omitted them from further steps of our study.

3.1.2. [^{35}S]GTPγS binding assay studies

Both DEX (a) and FLD (b) elevated [^{35}S]GTPγS binding in a concentration-dependent manner in non-pregnant tissues (Fig. 6a and b). MIF pre-treatment shifted to the right by inhibiting this elevation, which means less activation of G-proteins.

3.1.3. cAMP study

Uterine cAMP levels in non-pregnant uteri rose in the presence of both high and moderate dose of DEX and FLD moreover, this rise was inhibited significantly by pre-treatment with MIF (Fig. 7a and b)

3.1.4. In vivo study

The injection of one high dose of DEX led to the inhibition of contraction for non-pregnant animals, while FLD had no significant action. In addition, we observed that half an hour of pre-treatment with MIF ceased the inhibitory effect of DEX, however the difference between the effects DEX and DEX + MIF was not significant. Furthermore, MIF alone did not affect the uterus (Fig. 8a and b)
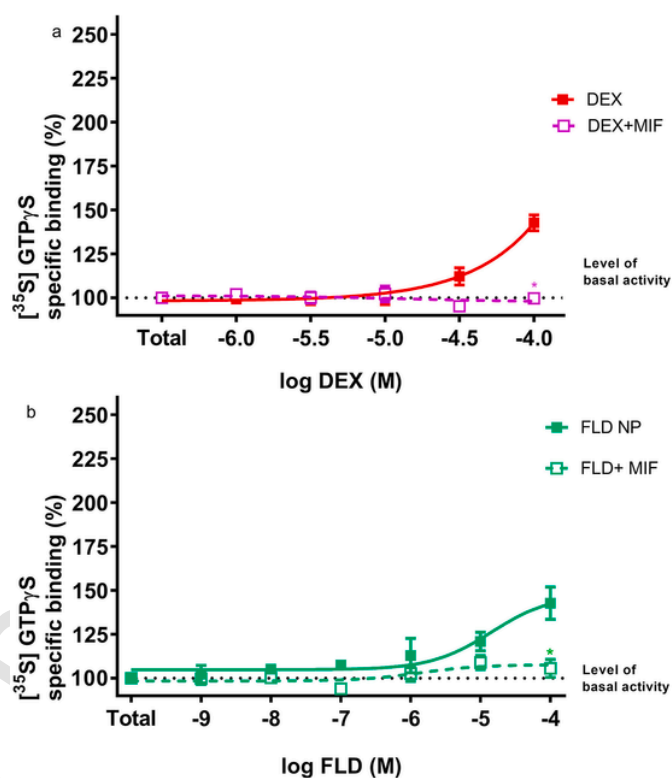

Fig. 6. Effect of dexamethasone (a) and fludrocortisone (b) on [^{35}S]GTPγS binding with or without pre-treatment with mifepristone in non-pregnant tissue. Mifepristone reduced [^{35}S]GTPγS binding which induced by dexamethasone(a) or fludrocortisone (b) uterine tissues. Basal activity (100%) refers to the level of [^{35}S]GTPγS binding without any substances. *: $p < 0.05$; DEX, dexamethasone; FLD, fludrocortisone; MIF, mifepristone.

3.2. Pregnant uterus

3.2.1. Isolated organ bath studies

DEX elicited a moderate relaxing effect (33%) at high concentration (10^{-4} M) in pregnant uteri which was slightly less compare with non-pregnant (Fig. 9 a, Fig. 10a). FLD showed a similar relaxing action (31%) only at the highest dose (10^{-4} M) (Fig. 9 d, Fig. 10b). FLD action did not change with pre-treatment of SPR or MIF Fig. 9e and f, Fig. 10b). In the other hand, while low dose of MIF (10^{-8} M) did not alter the action, but the effect of DEX was shifted to the right and even changed to a slight contraction only in the presence of a higher dose of MIF (10^{-6} M) (Fig. 9b and c, Fig. 10a). Similarly, Actinomycin D did not alter the action (Fig. 10.), and endothelium removal had no effect on these actions either (data not shown).

3.2.2. [^{35}S]GTPγS binding assay studies

In presence of DEX, elevation of [^{35}S]GTPγS binding in a concentration-dependent manner was observed in pregnant tissue, similarly, MIF pre-treatment shifted to the right by inhibiting this elevation, which means less activation of G-proteins. [^{35}S]GTPγS binding was very faintly elevated only by FLD. However, MIF did not change this small elevation (Fig. 11a and b).

3.2.3. cAMP study

Uterine cAMP levels in pregnant uteri rose in the presence of a high dose of DEX (10^{-4} M), moreover, this rise was inhibited by pre-treatment with MIF. There was no significant change in cAMP level compared to the control for FLD in pregnant tissues, (Fig. 12.).
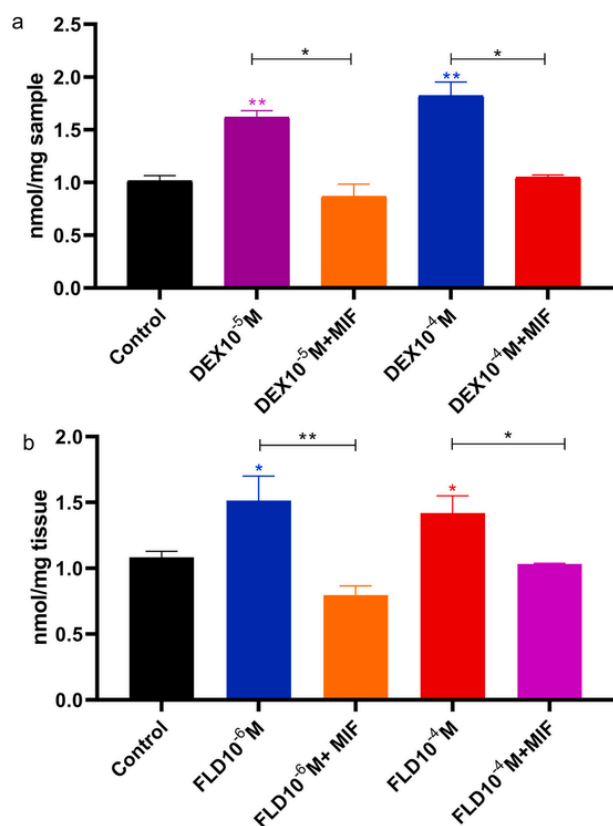
Fig. 7. Changes in the cAMP level of uterine non-pregnant tissues in the presence of dexamethasone (a) and fludrocortisone (b) alone and after pretreatment with mifepristone. The uterine cAMP level was expressed in nmol/mg tissue. *: $p < 0.05$; **: $p < 0.01$; MIF, mifepristone; FLD, fludrocortisone; DEX, dexamethasone.

3.2.4. In vivo study

The injection of one high dose of DEX led to the inhibition of contraction for pregnant animals, the action was stronger compare with non-pregnant ones. In addition, we observed that half an hour of pretreatment with MIF partially blocked the effect of DEX, furthermore, MIF alone did not affect the uterus significantly. FLD had no significant action on either animal group (Fig. 13).

4. Discussion

The non-genomic action of CSs and particularly GCs in uterine tissues inhibit induced contraction. The in vitro study of DEX showed a concentration-dependent relaxing effect, while FLD did not show such a relationship and had a slight moderate relaxing action only in a very high dose. In vivo experiments with DEX revealed a relaxing action which was more significant in pregnant animals. The results are similar to previous research about prompt GC action in the rat myocyte cell (Gong et al., 2016) as well as in the airway smooth muscles of guinea pigs (Wang et al., 2012) and mice (Sun et al., 2010). In contrast, FLD had no action at all in vivo, which may be explained by the weak water solubility of the compound and the subsequent limitation to administer high intravenous doses to non-pregnant rats. In the case of pregnant rats, we did not anticipate any in vivo effect after the lack of in vitro effectivity, which suggests that FLU cannot act through the non-genomic pathway in late pregnant uteri.

Since each experiment lasted a maximum of 30 min, genomic action was ruled out. As a further proof for non-genomic action, pre-treatment with genomic pathway blocker actinomycin D (DNA synthesis blocker) did not modify the action of steroid drugs, similarly to earlier studies (Gutiérrez et al., 1994; Mirdamadi et al., 2020; Panettieri et al., 2019).
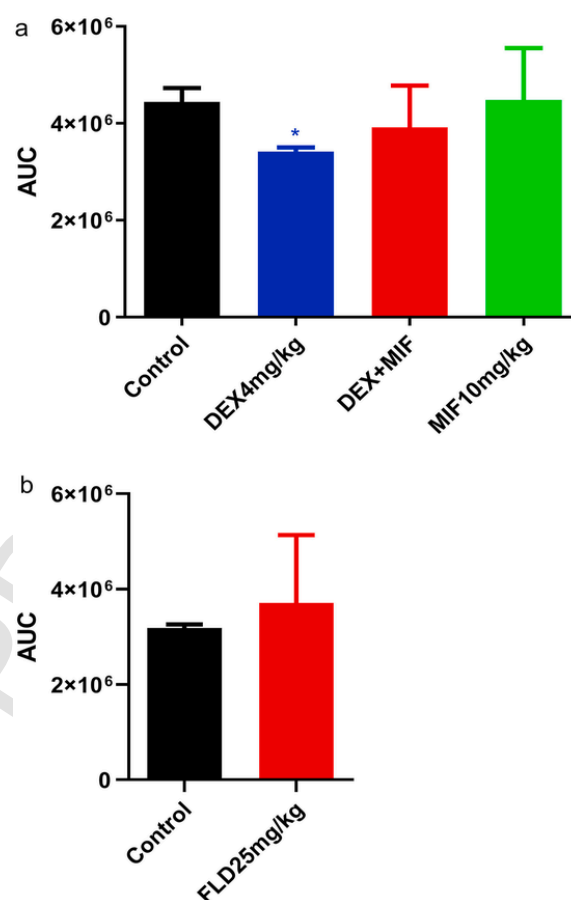

Fig. 8. Effect of dexamethasone and mifepristone (a) and fludrocortisone (b) on non-pregnant animals, alone and with pre-treatment with MIF in vivo, *: $p < 0.05$; DEX, dexamethasone; FLD, fludrocortisone; MIF, mifepristone.

In addition, endometrium removal did not alter the relaxing action of DEX of FLD, therefore, the measured effects are linked to myometrial steroid receptors. This finding is similar to our previous study about sex steroids (Mirdamadi et al., 2020), but is in contrast with earlier studies of airway smooth muscles, where the prompt relaxing action of CSs is endothelium-dependent (Horvath, 2006). This suggests the different functions of the endometrium and the endothelium in the uterus and lungs, respectively.

The prompt effect can be mediated by a non-specific interaction between the ligand and the cell membrane or by a specific interaction with the cytosolic receptor. Since SPR (the specific MC receptor antagonist) did not change the action of FLD, the role of MR at both membrane and cytosolic sites was ruled out, similarly to previous results (Sun et al., 2006). Furthermore, MIF in a low concentration (10^{-8} M, acting as a GC receptor antagonist) (Attardi et al., 2004) did not inhibit the prompt action of DEX, suggesting that GR has no non-genomic action in the uterus. Others have also shown that the non-genomic action of GCs is insensitive to GR because receptor blockade did not modify the action (Sun et al., 2006). However, besides its genomic action, the GR receptor may act through a non-genomic action as well in the airway smooth muscle (Wang et al., 2012). In our recent study, MIF in a high concentration (10^{-6} M, progesterone receptor antagonist) significantly inhibited the prompt relaxing action of DEX both in pregnant and non-pregnant tissues, and that of FLD in non-pregnant uteri. The same concentration has also been reported to inhibit the relaxing effect of budesonide in the skeletal muscle of mice (Pérez et al., 2013). Previously, we also found that MIF can inhibit the prompt action of sex steroids by reducing cAMP levels (Mirdamadi et al., 2020). Hence, we hypothesized that the acute effect of GC may be either GR-dependent in certain tis-
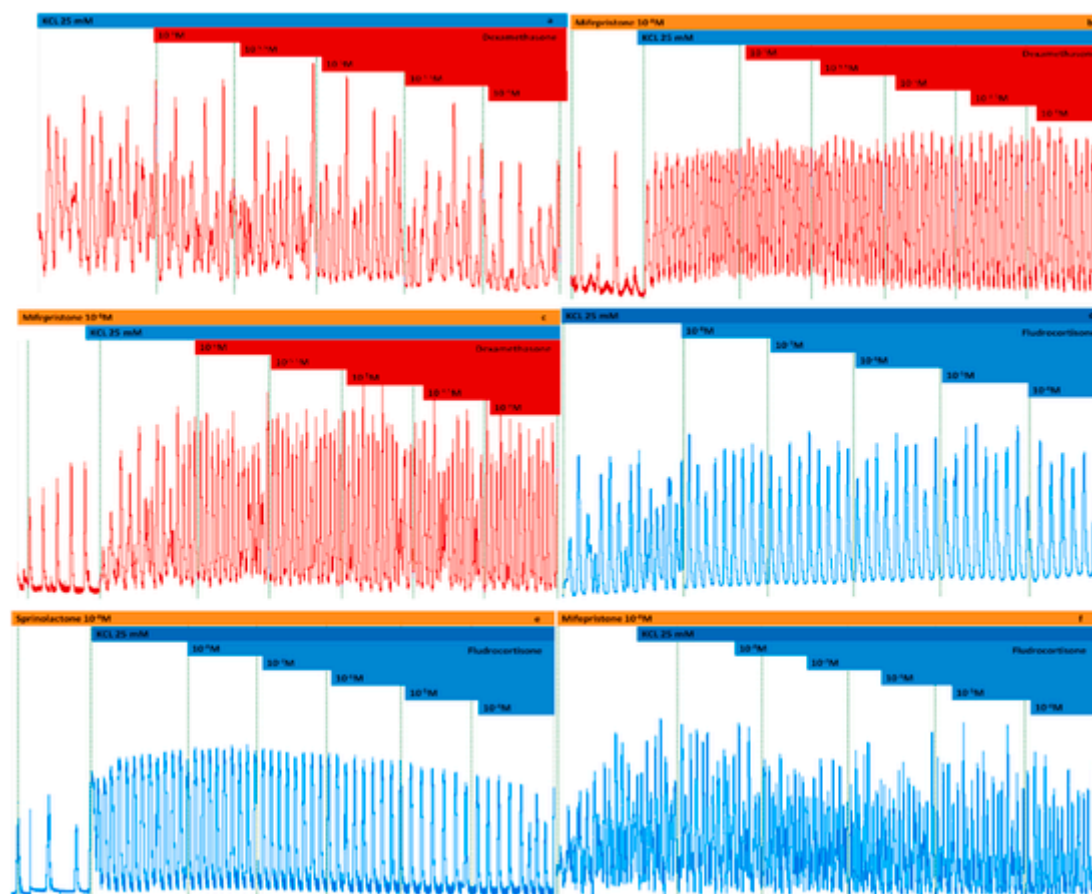
Fig. 9. Changes in KCL induced (25 mM) pregnant uterine contraction by dexamethasone (10^{-6} -10^{-4} M) (a) and fludrocortisone (10^{-8}- 10^{-4} M) (d). the effects were investigated in presence of mifepristone 10^{-8} M for dexamethasone(b), spironolactone 10^{-6} M for fludrocortisone (e) and mifepristone 10^{-6} M for both drugs (c and f). Each figure is a representative record.

sues such as airway smooth muscles (Panettieri et al., 2019) or GR-independent in the uterine tissue, blocking other possible pathways.

These effects might be mediated by GPCRs, especially the G-protein estrogen receptor (GPER), as it was reported previously (Han et al., 2013). A study reported that the relaxing action of aldosterone in vascular endothelial cells is mediated through GPER (Gros et al., 2013). The elevation of GTP and later cAMP requires G_α stimulation, therefore the relaxing action may suggest this (Nuñez et al., 2020), and MIF may block this non-genomic pathway. Consequently, GPER can also inhibit uterine smooth muscle contraction as a non-genomic action. Besides this, the non-genomic pathway could be mediated through the G_i or $G_{q/11}$ pathway, which was reported for corticosterone in PC12 cells (Qiu et al., 1998) or hippocampal neurons, respectively (Panettieri et al., 2019). Since the GTPγS study reveals activated GTP only, G_i or $G_{q/11}$ can also be involved in the uterine pathway.

5. Conclusion

A single high dose of GCs might prevent premature delivery and extend the gestational period, while MCs are practically not useful for this purpose. Considering the fact that GCs are widely used in threatening premature birth to enhance surfactant secretion preventing respiratory distress syndrome (Mullan, 2018) they may have a further benefit in delaying the time of delivery and reducing the risk of prematurity. Despite of the possible side effects of GCs related to this indication (Carson et al., 2016), the possible steroid tocolytic therapy would be safe if we excluded the genomic effect and only trigger the non-genomic (rapid) pathways. To solve this problem, further basic experiments are needed to search for suitable steroid analogues or specific antagonists.

CRediT authorship contribution statement

Mohsen Mirdamadi : Investigation, Formal analysis, Writing – original draft. **Annamaria Schaffer :** Formal analysis. **Tamara Barna :** Investigation. **Reza Samavati :** Investigation. **Kálmán F. Szűcs :** Formal analysis. **Edina Szűcs :** Investigation. **Sándor Benyhe :** Investigation, Formal analysis. **Mihály Szécsi :** Investigation. **Róbert Gáspár :** Conceptualization, Methodology, Writing – review & editing, Supervision.

Data availability

Data will be made available on request.
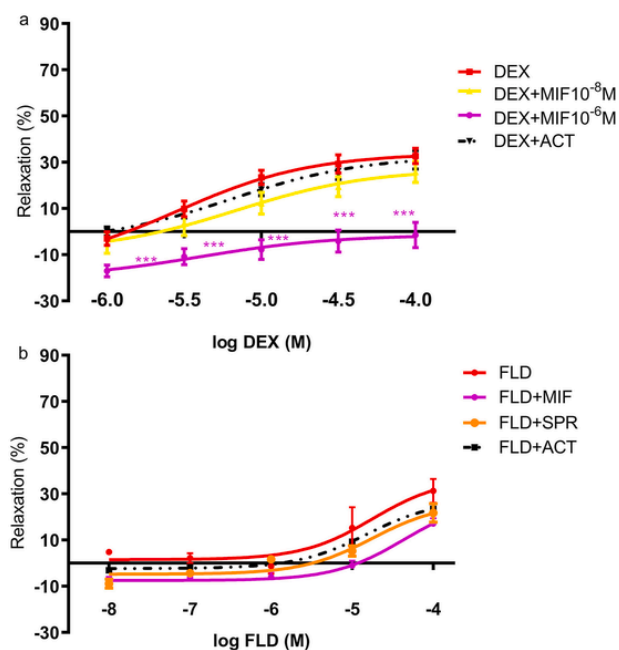
Acknowledgment

Fig. 10. Effects of dexamethasone(a) and fludrocortisone(b) on pregnant uterine contractions in vitro stimulated with KCl (25 mM) and pre-treated with spironolactone(10^{-6} M) for fludrocortisone or mifepristone (10^{-8} M) for dexamethasone, and mifepristone (10^{-6} M) for non-genomic steroid effects, Actinomycin D for both drugs separately, presented as a percentage of relaxation. ***: $p < 0.001$; ACT, Actinomycin D; DEX, dexamethasone; FLD, fludrocortisone; MIF, mifepristone.
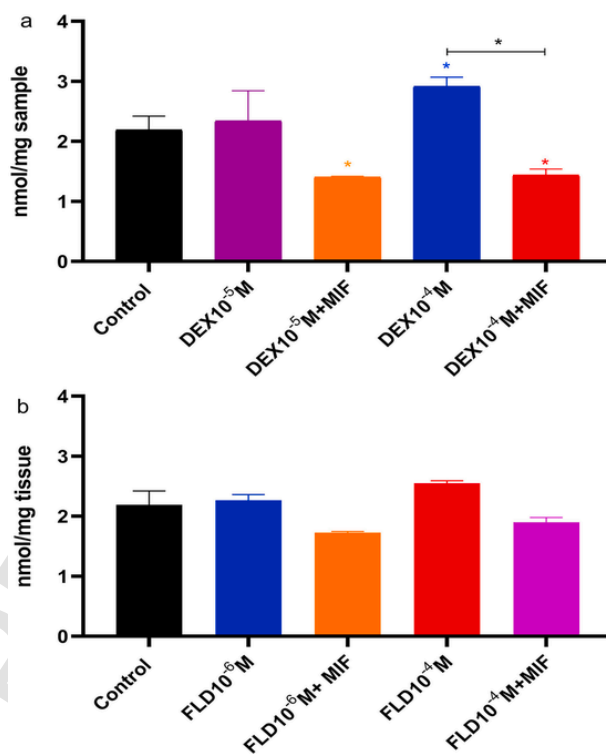

Fig. 12. Changes in the cAMP level of uterine pregnant tissues in the presence of dexamethasone (a) and fludrocortisone (b) alone and after pre-treatment with mifepristone. The uterine cAMP level was expressed in nmol/mg tissue. *: $p < 0.05$; DEX, dexamethasone; FLD, fludrocortisone; MIF, mifepristone.
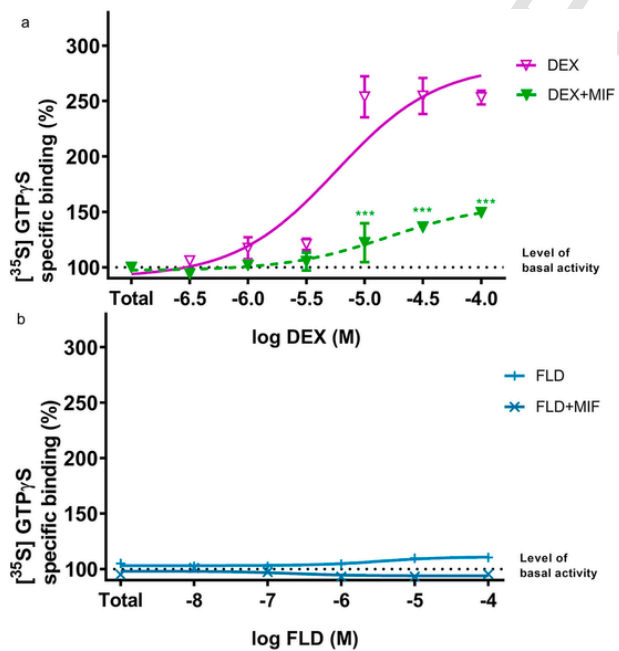

Fig. 11. Effect of dexamethasone (a) and fludrocortisone (b) on [^{35}S]GTPγS binding with or without pre-treatment with mifepristone in pregnant tissue. Mifepristone reduced [^{35}S]GTPγS binding which induced by dexamethasone(a) but fludrocortisone (b) did not show any effect. Basal activity (100%) refers to the level of [^{35}S]GTPγS binding without any substances. ***: $p < 0.001$; DEX, dexamethasone; FLD, fludrocortisone; MIF, mifepristone.
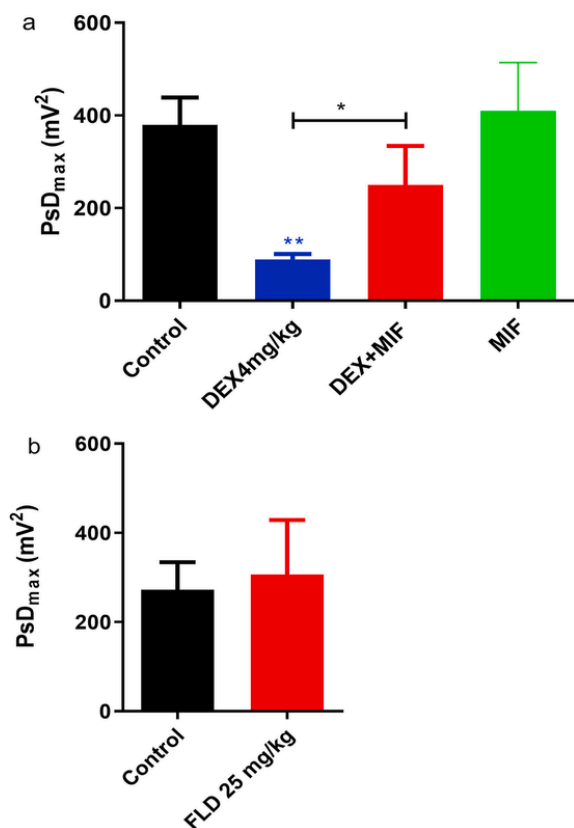
Fig. 13. Effect of dexamethasone and mifepristone (a) and fludrocortisone (b) on pregnant animals, alone and with pre-treatment with MIF, in vivo, *: p < 0.05; **: p < 0.01; DEX, dexamethasone; FLD, fludrocortisone; MIF, mifepristone.

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