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THE ASSESSMENT OF A SELECTIVE INHIBITION OF POTASSIUM CHANNELS AND GUANYLATE CYCLASE IN THE RELAXATION INDUCED BY EXOGENOUS NITRIC OXIDE IN THE HUMAN NONPREGNANT MYOMETRIUM

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To evaluate the involvement K^+ channels in the relaxation induced by exogenous nitric oxide, after preincubation with L-arginine analogue L-NA in the human nonpregnant myometrium. The activity of myometrial strips, obtained from 60 premenopausal hysterectomised women, mounted in an organ bath was recorded under isometric conditions using force transducers with digital output. Concentration-response curves to DEA/NO after inhibition of endogenous NO were constructed in the absence and presence of soluble granulate cyclase and K^+ channels' blockers. The responses were quantified by calculating the area under the curve, the amplitude and frequency of the contractions. The inhibition of NOS results in slight but significant attenuation of the myometrium strips response to DEA/NO. Pre-treatment with both sGC inhibitors after preincubation with L-NA did not counteract the DEA-NO-induced relaxation of the spontaneous contractions of the myometrial strips. Application of blockers of different types of K^+ channels to the myometrial strips significantly attenuated relaxing effect of cumulative DEA/NO administration in all cases. The present data indicate that even when endogenous production of NO is inhibited, the DEA/NO induced relaxation of human non-pregnant myometrium without involving the cGMP pathway.

INTRODUCTION

Nitric oxide (NO) is a potent relaxant of smooth muscles (Al-Azemi *et al.*, 2009; Palmer *et al.*, 1987), including its determinant role in the control of uterine contractility (Hoffmann *et al.*, 2003; Kuenzli *et al.*, 1998; Poniedzialek-Czajkowska *et al.*, 2011; Yallampalli *et al.*, 1993a). Thus, NO is the substance, that potentially may be exploited in suppressing the hyperactivity of myometrial contractions e.g. potential treatment of dysmenorrhoea (Doubova *et al.*, 2007; Wetzka *et al.*, 2001). NO is produced by uterine smooth muscle cells, from its precursor L-arginine, via NO synthases (NOS) (Ramsay *et al.*, 1996; Telfer *et al.*, 1995). However, the possible mechanism of action of NO in the uterus remains unclear. There are reports supporting a NO-cGMP dependent pathway (Izumi and Garfield, 1995; Jun *et al.*, 2003) and a NO-cGMP -independent pathway as well (Bolotina *et al.*, 1994; Modzelewska *et al.*, 2003a). A number of studies on both vascular and uterine smooth muscle have provided evidence for the involvement of potassium (K^+) channels in relaxation induced by NO donors (Khan *et al.*, 2009; Lindeman *et al.*, 1994). Our previous investigations have suggested that K^+_{ATP} channels could also be involved in NO-induced inhibition of spontaneous contractile activity of the non-pregnant human myometrium (Modzelewska *et al.*, 1998). Moreover, it has been reported that, beside Ca^{2+} and voltage dependent charybdotoxin-sensitive (CTX-sensitive) K^+ channels, apamin-sensitive K^+ channels are also present in the human both pregnant and non-pregnant myometrium (Modzelewska *et al.*, 2003a; Modzelewska *et al.*, 2003b).

N^G-nitro-L-arginine methyl ester (L-NAME), N^G-monomethyl -L-arginine (L-NAMA) and other L-arginine analogs inhibit NO production and relaxation in the vascular tissue trough blocking NOS (Fulep *et al.*, 2000; Osol *et al.*, 2009). Application L-NAME or N^G-nitro-L-arginine (L-NA) to the uterine muscle bath caused an increase in the contractile activity of the pregnant and non-pregnant myometrium (Buhimschi *et al.*, 1995; Yallampalli *et al.*, 1993b) indicating that endogenous NO is an important substance that regulates spontaneous contractile activity of the human myometrium. The current studies were designed to evaluate the involvement of K^+ channels in the relaxation induced by exogenous nitric oxide, after preincubation with L-arginine analogue L-NA in the human nonpregnant myometrium.

MATERIAL AND METHODS

Human uterine tissues were collected from 60 non-pregnant premenopausal women (aged 36-51 years; median 43 ± 4.2 years) undergoing hysterectomy because of dysfunctional bleeding, benign uterine tumors or cervical malignancy and being operated on during the follicular phase of the menstrual cycle. The women were informed about the nature and procedure of the study and gave their written consent. The local ethics committee approved the study.

Myometrial samples were excised transversally from the fundus of the uterus, placed in an ice-cold physiological salt solution and immediately transferred to the laboratory, where they were processed as previously described (Kostrzewska *et al.*, 2000; Modzelewska *et al.*, 2003b). Briefly, 4-8 strips, 6-7 mm in length and 2x2 mm in cross-section area were obtained under a dissecting microscope. The strips were then mounted in an organ bath containing 20 ml of physiological salt solution at 37ºC, pH 7.4, and bubbled with carbogen (95% O_2 + 5% CO_2). The strips were left for an equilibration period of 1-2 hours. During that period, the passive tension was adjusted to 3 mN.

The activity of the myometrium was recorded under isometric conditions by means of force transducers with digital output. The level of spontaneous contractile activity before the addition of DEA/NO was treated as a control level. The responses were quantified by calculating the area under the curve (AUC), and the amplitude and frequency of the contractions. The area was measured from the basal tension over a 10-min period after each stimulus. The effects were evaluated by comparing the experimental responses with the controls (set as 100%). The current studies were performed after 20 min preincubation with L-arginine analog N^G-nitro-L-arginine (L-NA) ($3 \cdot 10^{-4}$ mol/L) to inhibit endogenous NO production (Izumi *et al.*, 1995). Diethylamine-nitric oxide (DEA/NO), which was shown previously to inhibit spontaneous myometrial activity in human non-pregnant (Buhimschi *et al.*, 1995; Modzelewska *et al.*, 2003b) and pregnant (Modzelewska *et al.*, 2003a) or rat (Okawa *et al.*, 1999) myometrium, in a concentration-dependent manner, was used as a NO donor. As far as possible, experiments were performed with strips from the same uterus and were studied in parallel. Appropriate controls were run under similar experimental conditions obtained from the same woman. Concentration-response curves to DEA/NO (10^{-8}-10^{-4} mol/L, every 10 min) were constructed in the absence and presence of soluble guanylate cyclase (sGC) blockers: $5x10^{-6}$ mol/L methylene blue (Modzelewska *et al.*, 1998) or $5x10^{-3}$ mol/L cystamine (Modzelewska *et al.*, 2003b) and K^+ channels' blockers: 10^{-9} mol/L charybdotoxin (CTX) (Modzelewska *et al.*, 2003b), 10^{-8} mol/L apamin (Modzelewska *et al.*, 2003b) or $1.5 \cdot 10^{-6}$ mol/L glybenclamide (Modzelewska *et al.*, 1998). The incubation time for each blocker was 20 min. Only one concentration-response curve was performed in each uterine strip.

Chemicals

DEA/NO, purchased from Sigma Chemical Company, was dissolved in 0.01 M NaOH, and kept cold until dilution with cold pH 7.4 buffer immediately before addition to a bathing medium [27]. The concentration of NaOH in the organ bath never exceeded 0.001% v/v and had no influence on the experimental responses. Methylene blue, apamin, charybdotoxin (CTX), N^G-nitro-L-arginine (L-NA), and cystamine, purchased from the Sigma Chemical Company, were dissolved in distilled water. Glybenclamide was dissolved in DMSO. All the substances were added directly to an organ bath containing a physiological salt solution composed of (mmol/L): NaCl 136.9; KCl 2.68; $MgCl_2$ 1.05; NaH_2PO4 1.33; $CaCl_2$ 1.80; $NaHCO_3$ 25.0; and glucose 5.55.

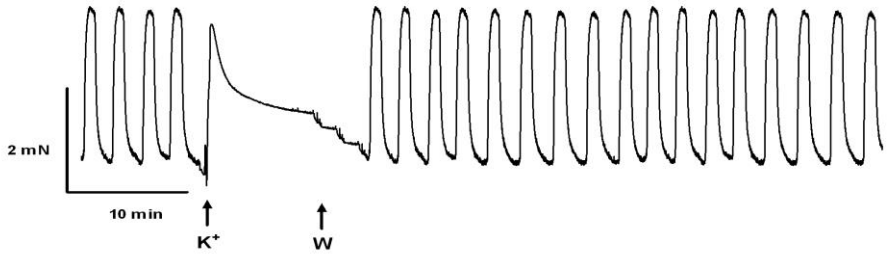

Fig. 1 Original recordings showing the typical spontaneous contractile activity of the human non-pregnant myometrium and the response to depolarisation caused by the solution containing high concentration of K^+; W – wash-out.

Statistical analysis

The data obtained was analyzed statistically using ANOVA or the Wilcoxon matched pairs signed rank test, where appropriate (PRISM 3.0 GraphPad Software Inc., San Diego, Calif.). The statistical significance was considered when the probability value was $P < 0.05$.

Throughout the paper, all results are expressed as mean ± S.E.M., and n denotes the number of tissues obtained from different patients.

RESULTS

All the experiments were performed on myometrial strips exhibiting regular, spontaneous contractile activity after equilibration (Fig. 1). The mean frequency of contraction was 3.95±0.3 per 10 min and its mean amplitude was 6.06±0.46 mN (n=30).

The effects of NOS inhibition on myometrial spontaneous contractions

Pre-treatment with $3 \cdot 10^{-4}$ mol/L L-NA (Izumi *et al.*, 1995) (n=10) caused an increase in the spontaneous contractile activity of myometrial strips (Fig. 2) demonstrated as a statistically significant increase of the area under the curve (AUC) (Fig. 3). This effect involved a significant increase in the basal tension, the mean frequency and amplitude of contractions (Fig. 3).

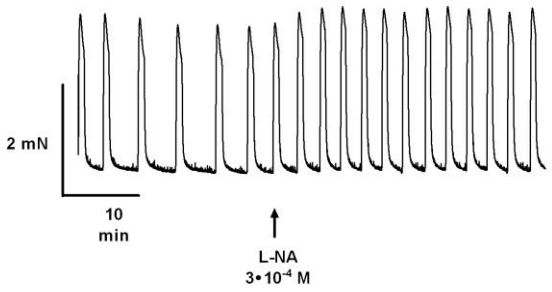

Fig. 2 Original recordings showing the typical effects of a NOS inhibitor, L-NA on the spontaneous contractile activity of the human non-pregnant myometrium. L-NA was added at the arrow.

The effects of sGC and K^+ channels inhibition on myometrial spontaneous contractions

Both inhibitors of sGC, methylene blue and cystamine, did not remarkably alter spontaneous myometrial activity with or without NOS inhibition, although after preincubation with L-NA, methylene blue caused the significant increase in the mean frequency (Tab.1).

Charybdotoxin (10^{-7} mol/L), a blocker of Ca^{2+}-sensitive K^+ channels with large conductance (BK[Ca]) caused no change of AUC, amplitude, and frequency of the spontaneous contractions but after NOS inhibition a small but nevertheless statistically significant decrease of AUC and amplitude of contractions was observed (Tab. 1).

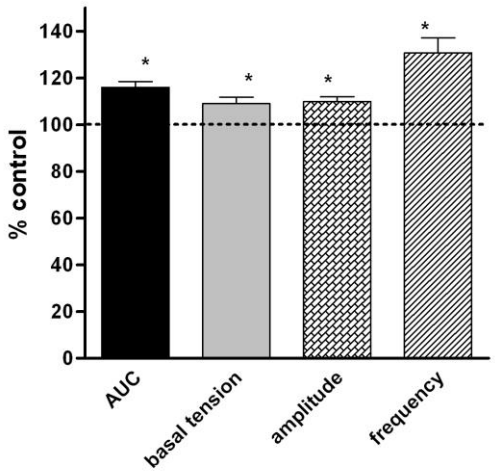

Fig. 3 Changes in the AUC, basal tension, amplitude and frequency of spontaneous contraction of the human non-pregnant myometrium after preincubation with a NOS inhibitor, L-NA ($3 \cdot 10^{-4}$ mol/L) (n=40). The data represent the means ± SEM. Spontaneous contractions of the myometrial strips were treated as a control.
* - $p < 0.05$

Apamin, a blocker of Ca^{2+}-sensitive K^+ channels with small conductance (SK[Ca]) at a concentration of 10^{-8} mol/L did not alter spontaneous myometrial contractions. In the presence of apamin, the mean values of amplitude and frequency of contractions did not differ significantly from those observed before the SK blocker administration (Tab. 1).

Table 1. The effect of sGC and K^+ channels blockers on the AUC, the mean amplitude and the mean frequency of myometrial contractions without and after preincubation with L-NA. The results are presented as the mean ± SEM of *n* experiments.

Channels blockers	AUC		Amplitude		Frequency	
	without L-NA	with L-NA	without L-NA	with L-NA	without L-NA	with L-NA
Cystamine	102.4±2.45	99.22±2.05	93.43±3.98	103.0±4.52	110.9±8.26	99.71±4.73
Methylene blue	101.7±2.96	102.4±1.41	98.63±2.79	97.6±3.89	100.01±0.25	114.5±6.13†
Apamin	99.57±2.26	89.76±3.59†‡	99.76±0.42	97.07±2.08	100.5±10,6	97.05±4.52
CTX	101,1±1.14	94.15±1.74†‡	101.1±1.82	96.63±1.57‡	102.2±2.51	103.7±8.63
Glybenclamide	102.4±3.48	87.54±2.93†‡	90.47±4.14*	89.08±2.18†	120.2±6.19*	87.19±4.13†‡

* - $p<0.05$ versus spontaneous contactility
† - $p<0.05$ versus spontaneous contactility after inhibition with L-NA
‡ - $p<0.05$ versus effects of blockers without inhibition with L-NA

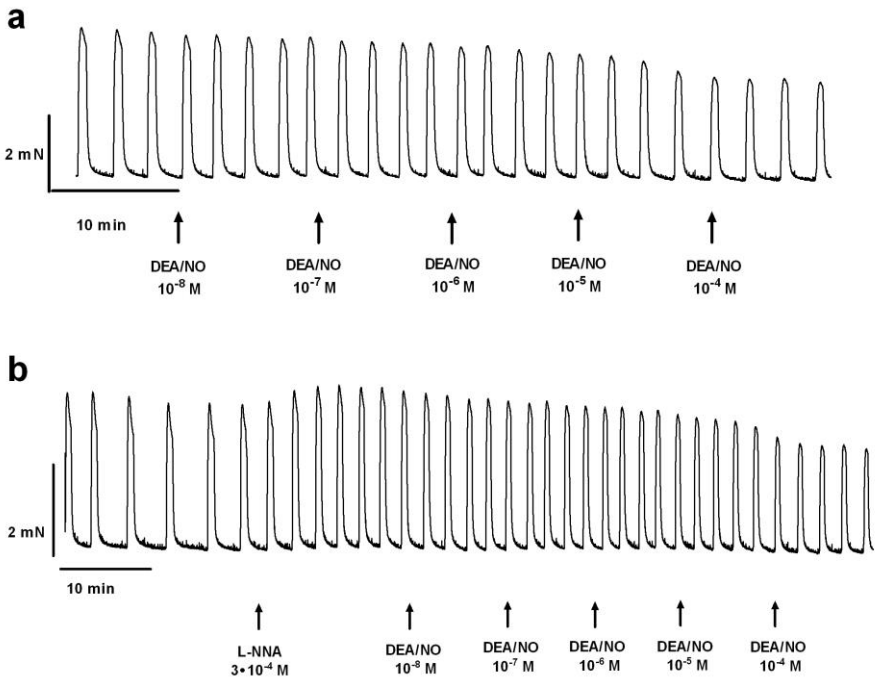

Fig. 4 Recordings showing the typical effects of cumulative administration of DEA/NO on tissue (a) without any blocker and (b) pretreated with $3 \cdot 10^{-4}$ mol/L L-NA. DEA/NO and L-NA were added at the arrows.

Although the addition of $1.5 \cdot 10^{-6}$ mol/L glybenclamide, a K^+_{ATP} channel blocker caused significant decrease in the mean amplitude of contractions and significant augmentation in its frequency no difference in the AUC measurements were observed (Tab. 1).

The effects of L-NA on DEA/NO-induced relaxation of myometrial spontaneous contractile activity

Cumulative administration of DEA/NO caused a concentration-dependent inhibition of the spontaneous activity of the myometrium strips (Fig. 4a). This effect was seen as a gradual decrease in both the mean amplitude (significant for concentrations of DEA/NO $\geq 10^{-5}$ mol/L) (Fig. 5a) and the mean frequency of the contractions, significant for 10^{-7} mol/L DEA/NO (Fig. 5b). Pre-treatment with L-NA, a NOS inhibitor did not counteract the DEA/NO-induced relaxation of the spontaneous contractions of the myometrial strips (Fig4b) and did not alter significantly the reduction in the mean frequency and changes in the mean amplitude (Fig. 5) as for the effect of DEA/NO alone. Moreover, the concentration response curve for AUC indicated that the log IC_{50} value for DEA/NO cumulative

Table 2. Log IC_{50} and E_{max} (the maximum relaxant response expressed as a percentage of the contractile activity before DEA/NO administration) for DEA/NO with or without preincubation with L-NA and in the presence of sGC blockers or K^+ channels blockers. *n* indicates the number of tissues. The results are presented as the mean ± SEM of *n* experiments.

Compound	n	LogIC_{50}±SEM	E_{max}±SEM (%)
DEA/NO	10	-6,24 ± 0,25	45,28 ± 7,89*
DEA/NO + L-NA	10	-5,97 ± 0,18	32,04 ± 2,75*†
DEA/NO + L-NA + cystamine	10	-5,96 ± 0,34	29,69 ± 6,91*
DEA/NO + L-NA + methylene blue	10	-6,15 ± 0,38	30,10 ± 7,32*

***** - $p<0.05$ versus spontaneous contactility
† - $p<0.05$ versus effects of DEA/NO alone
‡ - $p<0.05$ versus effects of DEA/NO after preincubation with L-NA

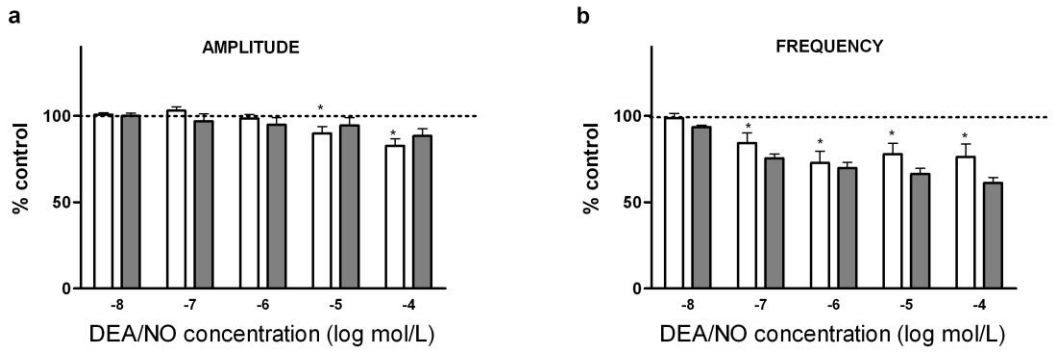

Fig. 5 The effect of 10^{-8}-10^{-4} M DEA/NO on (a) the mean amplitude and (b) the mean frequency of myometrial contractions without any blocker ☐ and after preincubation with L-NA ▒. The data represent the means ± SEM. Spontaneous contractions of the myometrial strips before adding DEA/NO were treated as a control. The asterisks indicate values significantly different from the control values ($p < 0.05$).

administration was not changed by preincubation of the strips with L-NA (Tab. 2). The difference was statistically non-significant. However, preincubation of the strips with L-NA resulted in considerable, statistically significant, attenuation of the maximum relaxant response (E_{max}) to DEA/NO (Tab. 2 and Fig. 6a).

The influence of sGC blockers on DEA/NO-induced relaxation of myometrial strips after preincubation with L-NA

The presence of $5 \cdot 10^{-6}$ mol/L methylene blue in the medium augmented the DEA/NO induced decrease in the amplitude of the contractions statistically significant in the DEA/NO concentration higher than 10^{-6} mol/L . Preincubation with methylene blue, did not change the frequency reduction caused by the DEA/NO in concentration range of 10^{-8}-10^{-6} mol/L but in the higher DEA/NO concentration the significant increase in the mean frequency was observed with reference to DEA/NO effects without inhibition of endogenous NO production (Fig. 7b).

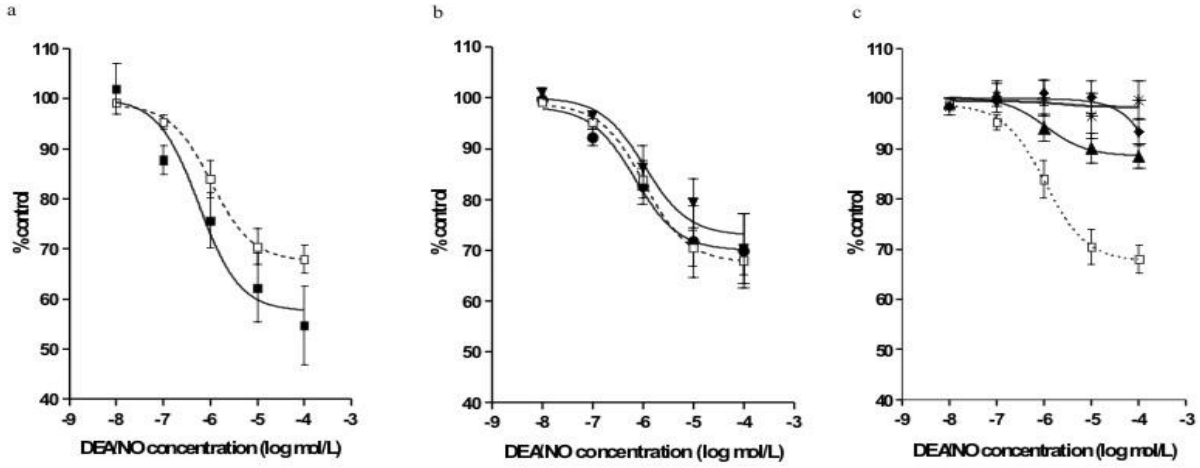

Fig. 6. The effect of 10^{-8}-10^{-4} M DEA/NO on the spontaneous contractile activity of the human non-pregnant myometrium (a) without any blocker (■) and after preincubation with L-NA (❒); (b) after NOS inhibition (L-NA) without any sGC blocker (❒), after preincubation with methylene blue (●) or cystamine (▼); (c) after NOS inhibition without any K^+ channel blocker (❒), after preincubation with CTX (✳), apamine (◆) and glybenclamide (▲). Spontaneous contractions of the myometrial strips before the addition of DEA/NO were treated as a control. The data represent the means ± SEM

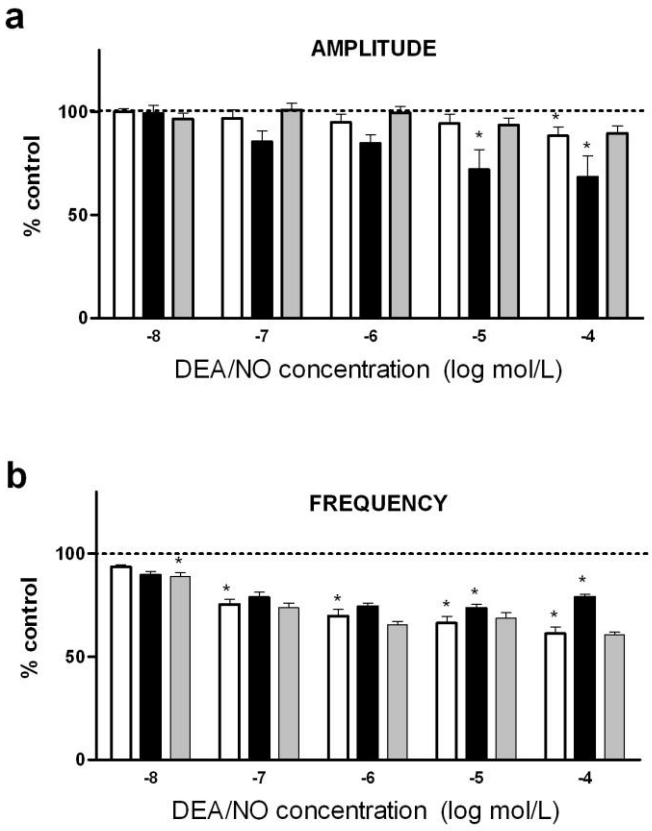

Fig. 7. The effect of 10^{-8}-10^{-4} M DEA/NO on (a) the mean amplitude and (b) the mean frequency of contractions after preincubation with L-NA without any sGC blocker ☐, after preincubation with methylene blue ■ and cystamine ▭. The data represent the means ± SEM. Spontaneous contractions before adding DEA/NO were treated as a control in case of myometrial strips without any sGC blocker. When myometrial strips were preincubated with methylene blue or cystamine effects of the appropriate DEA/NO concentration were treated as a control. * - $p < 0.05$ Doted lines indicates mean values observed in untreated strips.

After preincubation with L-NA, pre-treatment with $5 \cdot 10^{-3}$ mol/L cystamine (a sGC blocker) did not abolish the DEA-NO-induced relaxation of the spontaneous contractions of the myometrial strips (Fig. 6b). The presence of cystamine did not significantly alter the reduction in the mean amplitude induced by DEA/NO without incubating with L-NA (Fig. 7a). The significant decrease of the mean frequency was observed only in the lowest concentration of DEA/NO used (Fig. 7b).

The analysis of the AUC, $\log IC_{50}$ and E_{max} showed that inhibition of sGC activity did not prevent DEA/NO-induced inhibition of the spontaneous contractions of the myometrial strips (Fig. 6 and Tab. 2).

The influence of K^+ channels blockers on DEA/NO-induced relaxation of myometrial strips after inhibition of NOS

The effect of K^+ channels blockers on NO-induced relaxation of the myometrium strips has been studied in presence of $3 \cdot 10^{-4}$ mol/L L-NA (Fig. 6c). In this group of experiments, charybdotoxin (CTX), apamin and glybenclamide were used to block BK[Ca], SK[Ca] and K^+_{ATP} channels, respectively. On the basis of AUC estimation it was stated that in presence of any of the three blockers in the medium, the relaxing effect of cumulatively administrated DEA/NO on myometrium strips was significantly attenuated.

In strips preincubated with L-NA, CTX completely inhibited the myometrium response to DEA/NO, in the range concentrations used in experiments with reference to DEA/NO effects without inhibition of endogenous NO production.

In the same conditions, apamin shifted the concentration – response curve to the right, toward the higher concentrations of DEA/NO. In both cases, evaluation of the IC_{50} and E_{max} values was impossible. In presence of L-NA, glybenclamide did not change significantly the log IC_{50} but significantly decreased the maximum response (E_{max}) to the cumulatively administered DEA/NO.

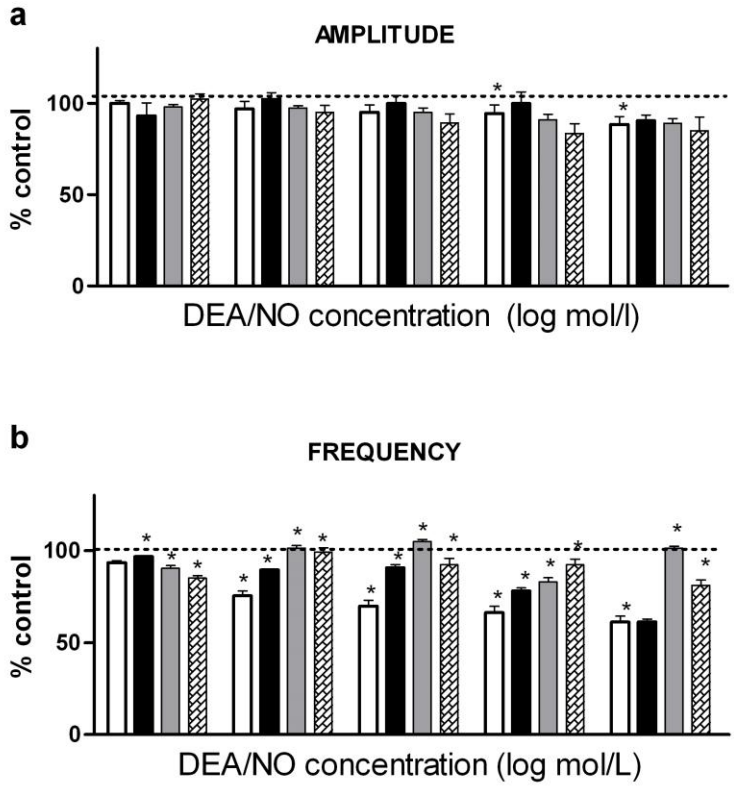

Fig. 8 The effect of 10^{-8}-10^{-4} M DEA/NO on (a) the mean amplitude and (b) the mean frequency of contractions after preincubation with L-NA without any K^+ channel blocker ☐, after preincubation with apamin ■, CTX ▒ and glybenclamide ▨. The data represent the means ± SEM. Spontaneous contractions before adding DEA/NO were treated as a control in case of myometrial strips without any K^+ channel blocker. When myometrial strips were preincubated with apamin, CTX or glybenclamide effects of the appropriate DEA/NO concentration were treated as a control. * - $p < 0.05$

In the presence of NOS inhibitor, preincubation with apamine did not change significantly the DEA/NO effect on the mean amplitude of contractions compared to the NO donor effect on myometrial strips without SK[Ca] channels blocking (Fig. 8a). However, statistically significant attenuation of the mean frequency decrease in the range from 10^{-7} to 10^{-5} mol/L DEA/NO was observed (Fig. 8b).

In experiments where CTX was used, there were not significant change in the mean amplitude of contractions while the frequency decrease was inhibited in all concentration of DEA/NO comparing to DEA/NO effects without inhibition of endogenous NO production (Fig. 8).

The inhibition of K^+_{ATP} channels caused concentration dependent augmentation of the amplitude decrease and statistically significant inhibition of the frequency decrease after 10^{-7} to 10^{-4} mol/L DEA/NO administration in relation to effects observed in strips without preincubation with glybenclamide (Fig. 8).

DISCUSSION

Nitric oxide is synthesized from the oxidative deamination of guanidine nitrogen of L-arginine by a family of enzymes known as NOS (Moncada and Higgs, 1993). This enzyme has been reported to exist in several isoforms, and at least two isoforms, constitutive and inducible, have been isolated (Bredt *et al.*, 1990) and cloned (Xie *et al.*, 1992). Convincing evidence for the presence of NOS has been provided by immunocytochemistry in uterus from pre- and post-menopausal women (Roberto da Costa *et al.*, 2007; Telfer *et al.*, 1995) as well as by biochemical analysis of NOS activity in both pregnant and non-pregnant women (Ramsay *et al.*, 1996). Our present data confirm that inhibition of NOS caused an increase in the spontaneous contractile activity of myometrial strips (Izumi *et al.*, 1995). The effect appears as a significant increase of the AUC as well as a significant rise in the basal tension, the mean frequency and amplitude of contractions. This observation suggests the presence of functional NOS in non-pregnant uterus and maintain that endogenous NO

is one of the main regulators of the non-pregnant uterine contractility.

Previous studies have demonstrated that different NO donors like S-nitroso-L-cysteine (CysNO) (Bradley *et al.*, 1998), sodium nitroprusside (SNP) (Hoffmann *et al.*, 2003; Lee *et al.*, 2008) or DEA/NO (Modzelewska and Kostrzewska, 2005; Modzelewska *et al.*, 2003b) relaxed the spontaneous contractions of human non-pregnant uterus.

The present data indicate that the inhibition of NOS results in slight but significant attenuation of the myometrium strips response to DEA/NO. In this group of experiments, the spontaneous contractile activity in presence of L-NA was treated as 100%. Thus, the enhancement of contractile activity caused by the inhibition of endogenous NO is excluded from the data. Therefore, this finding suggests that endogenous NO play a role in the response of human non-pregnant myometrium to DEA/NO. Further study are necessary to confirm or reject this observation.

Until now, the precise mechanisms by which NO causes relaxation of smooth muscle remains unclear. One postulated mechanism is that NO increases cGMP and relaxes smooth muscles via the inhibition of myosin light chain phosphorylation (Buxton, 2008; Cornwell *et al.*, 2001). Another possible mechanism is that increased cGMP levels lead to hyperpolarisation of the cellular membranes ((Khan *et al.*, 2009; Zygmunt and Hogestatt, 1996) resulting in smooth muscle relaxation.

We used methylene blue or cystamine to inhibit the sGC. Although, methylene blue is generally accepted as an antagonist of sGC (Moncada and Higgs, 1991), some authors consider this effect to be rather weak (Marczin *et al.*, 1992). It has been demonstrated that methylene blue not only acts as a direct inhibitor of NO synthase (Mayer *et al.*, 1993; Modzelewska and Kostrzewska, 2005) but is also a much less specific and potent inhibitor of sGC than previously assumed (Mayer *et al.*, 1993).

Pre-treatment with both sGC inhibitors after preincubation with L-NA did not counteract the DEA-NO-induced relaxation of the spontaneous contractions of the myometrial strips. Furthermore, their maximum relaxant response was significantly weaker when compared with DEA/NO cumulative administration alone while there was no significant difference when DEA/NO treated strips were preincubated with L-NA. Still, in the presence of cystamine E_{max} did not significantly differ from appropriate value when L-NA was no used whereas in the presence of methylene blue we observe significant difference between maximum relaxant responses with (59.52 ± 10.36) or without preincubation with L-NA (30.10 ± 7.33) (Modzelewska and Kostrzewska, 2005). However, the presence of cystamine or methylene blue significantly deepened the decrease in the mean amplitude induced by DEA/NO without changing the reduction in the mean frequency observed after cumulative DEA/NO administration comparing to effects of DEA/NO after preincubation with L-NA. Our results show that there are no significant impact on dose-response curve in both cases. So, these data indicate that even when endogenous production of NO is inhibited, the DEA/NO induced relaxation of human non-pregnant myometrium without involving the cGMP pathway.

Potassium channels activity is the main determinant of membrane potential in smooth muscle cells. Potassium efflux through opened K^+ channels causes hyperpolarisation resulting in the inhibition of the voltage-dependant Ca^{2+} channel opening and the promotion of the smooth muscle relaxation (Brayden, 1996; Danylovych and Danylovych Iu, 2007; Kostrzewska *et al.*, 1996). It has been reported that NO may activate K^+ channels directly, not involving the cGMP pathway (Kuenzli *et al.*, 1998; Modzelewska *et al.*, 1998). It was also shown that NO activates Ca^{2+}-activated K^+ channels in myometrial cells from human pregnant uterus using the patch-clamp technique (Khan *et al.*, 2009; Shimano *et al.*, 2000).

Our previous results showed that blockers of different types of K^+ channels inhibits the DEA/NO-induced relaxation of spontaneous activity of the pregnant (Modzelewska *et al.*, 2003a) and non-pregnant myometrium (Modzelewska *et al.*, 2003b; Modzelewska *et al.*, 1998). To explore the role of K^+ channels in NO-mediated relaxation of the human non-pregnant uterus we examined the involvement of K^+ channels in the relaxation induced by exogenous NO, in the human myometrium strips with inhibited the endogenous production of NO.

Application of blockers of different types of K^+ channels to the myometrial strips significantly attenuated relaxing effect of cumulative DEA/NO administration in all cases. In contrast to the effects mediated by sGC inhibitors, preincubation with apamine did not change significantly the DEA/NO effect on the mean amplitude of contractions but the statistically significant attenuation of the mean frequency decrease was observed. In experiments where CTX was used, also there were not significant changes in the mean contractions' amplitude while the frequency decrease was inhibited. In case of inhibition of K^+_{ATP} channels concentration dependent augmentation of the amplitude decrease and statistically significant inhibition of the frequency decrease in relation to effects observed in strips without preincubation with glybenclamide were observed. Moreover, comparing present results with our previous data (Modzelewska *et al.*, 2003b; Modzelewska *et al.*, 1998) we have not observe significant difference between the mean AUC value at the highest DEA/NO concentration in the bath medium

in the presence of all K^+ channels blockers used respectively.

Although, CTX or apamin alone did not influence the spontaneous contractility of myometrial strips but after preincubation with L-NA the considerable decrease of mean AUC value was observed. The lack of influence of these blockers on spontaneous contractions might suggest that BK (Garcia *et al.*, 1995) and SK channels do not participate substantially in the regulation of the spontaneous activity of the non-pregnant myometrium. Nevertheless, incubation with a NOS blocker disclosed their possible influence. In the presence of glybenclamide in both cases we noticed significant decrease in the mean amplitude of contractions and significant augmentation in its frequency, no differences in the AUC measurements were observed (Tab. 2). We presume that because the basal tone of tested strips remained stabile effects of the opposite changes in its mean amplitude and frequency canceled each other out.

CONCLUSIONS

Still, on the basis of presented results it is difficult to attempt to put a unquestionable hypothesis explaining the pathways of nitric oxide induced relaxation of the human nonpregnant myometrium. Taking into consideration the fact that inhibiting of endogenous NO synthesis changes reaction of muscles for sGC and K^+ channels blockers you may say that this is a complex process including the modification of structure properties which are responsible for a transport through the cell membranes. As the sustain of spontaneous contractility does not cause changes this may lead to the conclusion that the DEA/NO influence has no relation with guanylate cyclase and is independent from endogenous NO production.

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REFERENCES

Al-Azemi M., Refaat B., Amer S., Ola B., Chapman N., & Ledger W. (2009). *Fertil Steril* **94,** 833-840.

Bolotina V. M., Najibi S., Palacino J. J., Pagano P. J., & Cohen R. A. (1994). *Nature* **368,** 850-853.

Bradley K. K., Buxton I. L., Barber J. E., McGaw T., & Bradley M. E. (1998). *Am J Physiol* **275,** C1668-C1673.

Brayden J. E. (1996). *Clin Exp Pharmacol Physiol* **23,** 1069-1076.

Bredt D. S., Hwang P. M., Glatt C. E., Lowstein C., Reed R. R., & Snyder S. H. (1990). *Nature* **351,** 768-770.

Buhimschi I., Yallampalli C., Dong Y.-L., & Garfield R. E. (1995). *Am J Obstet Gynecol* **172,** 1577-1584.

Buxton I. L. (2008). *Proc West Pharmacol Soc.* **51,** 78-82.

Cornwell T. L., Li J., Sellak H., Miller R. T., & Word R. A. (2001). *J Clin Endocrinol Metab* **86,** 3981-3988.

Danylovych H. V., & Danylovych Iu V. (2007). *Ukr Biokhim Zh.* **79,** 30-37.

Doubova S. V., Morales H. R., Hernandez S. F., del Carmen Martinez-Garcia M., de Cossio Ortiz M. G., Soto M. A., Arce E. R., & Lozoya X. (2007). *J Ethnopharmacol.* **110,** 305-310. Epub 2006 Oct 2013.

Fulep E. E., Vedernikov Y. P., Saade G. R., & Garfield R. E. (2000). *Gen Pharmacol* **35,** 297-301.

Garcia M., Knaus H. G., Munujons P., Slaughter R., & Kaczorowski G. J. (1995). *Am J Physiol* **269,** C1-C10.

Hoffmann P., Stanke-Labesque F., Fanchin R., Dilai N., Pons J. C., & Ayoubi J. M. (2003). *Hum Reprod* **18,** 148-151.

Izumi H., & Garfield R. E. (1995). *Eur J Obstet Gynecol Reprod Biol* **60,** 171-180.

Izumi H., Makino Y., Shirakawa K., & Garfield R. E. (1995). *Am J Obstet Gynecol* **172,** 1477-1484.

Jun C. H., Lee T. S., & Sohn U. D. (2003). *Auton Autacoid Pharmacol* **23,** 159-166.

Khan L. H., Rosenfeld C. R., Liu X. T., & Magness R. R. (2009). *Am J Physiol Endocrinol Metab* **298,** 17.

Kostrzewska A., Laudański T., & Batra S. (1996). *Acta Obstet Gynecol Scand* **75,** 886-891.

Kostrzewska A., Modzelewska B., & Batra S. (2000). *Hum Reprod* **15,** 1927-1931.

Kuenzli K. A., Buxton I. L., & Bradley M. E. (1998). *Br J Pharmacol* **124,** 63-68.

Lee D. H., Joo B. S., Suh D. S., Park J. H., Choi Y. M., & Lee K. S. (2008). *Fertil Steril* **89,** 1514-1521.

Lindeman K. S., Fernandes L. B., Croxton T. L., & Hirshman C. A. (1994). *Am J Physiol* **266,** L232-L237.

Marczin N., Ryan U. S., & Catravas J. D. (1992). *J Pharmacol Exp Ther* **263,** 170-179.

Mayer B., Brunner F., & Schmidt K. (1993). *Biochem Pharmacol* **45,** 367-374.

Modzelewska B., Kleszczewski T., & Kostrzewska A. (2003a). *Cell Mol Biol Lett* **8,** 69-75.

Modzelewska B., & Kostrzewska A. (2005). *Cell Mol Biol Lett.* **10,** 389-400.

Modzelewska B., Kostrzewska A., Sipowicz M., T. K., & B. S. (2003b). *Reprod Biol Endocrinol* **1,** 8.

Modzelewska B., Sipowicz M., Saavedra J. E., Keefer L. K., & Kostrzewska A. (1998). *Biochem Biophys Res Commun* **253,** 653-657.

Moncada S., & Higgs E. A. (1991). *Eur J Clin Invest* **21,** 361-374.

Moncada S., & Higgs E. A. (1993). *N Engl J Med* **329,** 2002-2012.

Okawa T., Vedernikov Y. P., Saade G. S., Longo M., Olson G. L., Chwalisz K., & Garfield R. E. (1999). *Am J Obstet Gynecol* **181,** 649-655.

Osol G., Barron C., Gokina N., & Mandala M. (2009). *J Vasc Res.* **46,** 478-486. Epub 2009 Feb 2010.

Palmer R. M., Ferrige A. G., & Moncada S. (1987). *Nature* **327,** 524-526.

Poniedzialek-Czajkowska E., Marciniak B., Kimber-Trojnar Z., Leszczynska-Gorzelak B., & Oleszczuk J. (2011). *Current pharmaceutical biotechnology* **12,** 743-749.

Ramsay B., Sooranna S. R., & Johnson M. R. (1996). *Obstet Gynecol* **87,** 249-253.

Roberto da Costa R. P., Ferreira-Dias G., Mateus L., Korzekwa A., Andronowska A., Platek R., & Skarzynski D. J. (2007). *Domest Anim Endocrinol* **32,** 287-302.

Shimano M., Nakaya Y., Fukui R., Kamada M., Hamada Y., Maeda K., & Aono T. (2000). *Gynecol Obstet Invest* **49,** 249-254.

Telfer J. F., Lyall F., Norman J. E., & Cameron I. T. (1995). *Hum Reprod* **10,** 19-23.

Wetzka B., Schafer W. R., Stehmans A., & Zahradnik H. P. (2001). *Gynecol Endocrinol* **15,** 34-42.

Xie Q.-W., Cho H. J., Calaycay J., Mumford R. A., & Swiderek K. M. (1992). *Science* **256,** 225-228.

Yallampalli C., Garfield R. E., & Byam-Smith M. (1993a). *Endocrinology* **133,** 1899-1902.

Yallampalli C., Izumi H., Byam-Smith M., & Garfield R. E. (1993b). *Am J Obstet Gynecol* **170,** 175-185.

Zygmunt P. M., & Hogestatt E. D. (1996). *Br J Pharmacol* **117,** 1600-1606.