INFLUENCE OF MODULATORS OF RELAXANT EFFECT OF PENTOXYPHYLLINE IN ISOLATED RAT UTERUS

Jelena Kordic-Bojinovic[1], Dragana Jokanovic[1], Dragana Stankovic[1], Slobodan Jankovic[2] and Slobodan R. Milovanovic[1]
[1]Department of Pharmacology, Toxicology and Clinical Pharmacology,
Faculty of Medicine, University of East Sarajevo, Foca, Republic of Srpska, Bosnia and Herzegovina
[2]Department of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Kragujevac, Serbija

UTICAJ MODULATORA RELAKSANTNOG EFEKTA PENTOKSIFILINA NA IZOLOVANOM UTERUSU PACOVA

Kordić-Bojinović[1], Dragana Jokanović[1], Dragana Stanković[1], Slobodan Jankovićc[2] i Slobodan R. Milovanović[1]
[1] Odsek za farmaciju, toksikologiju i kliničku farmakologiju
Medicinski fakultet, Univerzitet u Istočnom Sarajevu, Foča, Republika Srpska, Bosna i Hercegovina
[2] Odsek za farmaciju, Medicinski fakultet Univerziteta u Kragujevcu, Srbija

Received / Primljen: 9. 6. 2010 . Accepted / Prihvaćen: 10. 7. 2010.

ABSTRACT

Background. *Pentoxyphylline is a methylxanthine derivative used in the treatment of peripheral vascular diseases. One effect of pentoxyphylline action is the vasodilatation of blood vessels. In this study, the effect of increasing concentrations of pentoxyphylline on contractility of isolated rat uteri was examined.*

Methods. *Uteri were isolated from virgin Wistar rats (180–220 g) and suspended in an isolated organ bath chamber containing De Jalon's solution and aerated with 95% O2 and 5% CO2. The temperature was maintained at 37°C. Isometric contractions were recorded using an isometric force transducer (Ugo Basile). The preload of the preparation was about 1 g. Uteri were allowed to contract spontaneously or in the presence of Ca2+ (6 mM) and were treated with pentoxyphylline.*

Results. *Pentoxyphylline caused concentration-dependent inhibition of spontaneous rhythmic uterine activity and uterine activity induced by calcium. We showed that the inhibitory effect of pentoxyphylline depends on the type of muscle contraction activation, and that it is significantly stronger in spontaneous contractions induced by calcium Ca2+. As opposed to methylene blue, L-arginine and glibenclamide did not antagonise the relaxing effect of pentoxyphylline on the isolated rat uterus.*

Conclusion. *Our results suggest that the signaling pathway by which pentoxyphylline causes relaxation of uterine muscle cells does not involve NO because the presence of L-arginine did not affect the action of the drug; however, it may depend on an NO-independent cGMP signaling pathway because the presence of methylene blue significantly antagonised the effect of pentoxyphylline. These results indicate that pentoxyphylline could be a potential tocolytic drug.*

Key words: *pentoxyphylline, rat uterus, L-arginine, glibenclamide, methylene blue*

Running title: *Pentoxyphylline Inhibits Contractility of Rat Uterus*

SAŽETAK

Cilj. *Pentoksifilin, koji se koristi za lečenje perifernih vaskularnih obolenja, je derivat metilksantina. Jedan od načina delovanja pentoksifilina je prouzrokovanje vazodilatacije krvnih sudova. U ovom radu ispitivali smo efekt rastućih koncentracija pentoksifilina na kontraktilnost izolovanog uterusa pacova.*

Metode. *Uterusi, koji su izolovani od neparenih ženki pacova Wistar soja (180-220 g),držani su u kupatilu za izolovane organe na temperaturi od 37°C, u De Jalon-ovom rastvoru kroz koji je propuštana mešavina od 95% kiseonika i 5% ugljendioksida. Izometrijske kontrakcije su registrovane korišćenjem izometrijskog transducera Ugo Basile, pri opterećenju preparata od 1 g. Efekt pentoksifilina je ispitivan na kontrakcije za vreme spontane ritmičke aktivnosti i u prisustvu kalcijuma (6 mM),*

Rezultati. *Pentoksifilin je prouzrokovao koncentracijski-zavisnu inhibciju spontane rithmičke aktivnosti, kao i fazne aktivnosti prouzrokovane kalcijumom. Inhibitorni efekt pentoksifilina zavisio je od tipa aktivacije glatkog mišića uterusa. On je ispoljio značajno jači relaksantni efekt na kontrakcije prouzrokovane kalcijumom. Nasuprot metilenskom plavilu, L-arginin i glibenklamid ne antagonizuju relaksantni efekt pentoksifilina na izolovanom uterusu pacova.*

Zaključak. *Dobijenii rezultati sugerišu da signalini putevi sa kojima pentoksifilin prouzrokuje relaksaciju glatkih mišičnih ćelija uterusa, za razliku izolovanih krvnih sudova, verovatno ne uključuje u većoj meri prisustvo NO (jer prisustvo L-arginine nije menjalo efekt ovog leka), ali zavisi od cGMP signalinih puteva nezavisnih od NO (zbog toga što prisustvo metilenskog plavila značajno antagonizuje njegov efekt). Ovi rezultatia ukazuju da bi pentoksifilin mogao da bude potencijalni tokolitički lek.*

Ključne reči: *pentoksifilin, uterus pacova, L-arginin glibenclamid i metilensko plavilo*

Kratki naslov: *pentoksifilin inhibiše kontrakcije uterusa pacova*

UDK 615.256 / Ser J Exp Clin Res 2010; 11 (3): 99-104

Correspondence to: Slobodan R. Milovanovic, M.D., Ph.D. / Department of Pharmacology, Toxicology, and Clinical Pharmacology, School of Medicine, University of East Sarajevo, Foca, Republic of Srpska, Bosnia and Herzegovina / E-mail: milarados@ptt.rs / Phone: + 387 651 560-504

INTRODUCTION

Tocolytics, such as β2-adrenergic agonists, are frequently used to prevent miscarriage and premature birth, however these compounds are associated with insufficient efficacy and excessive side-effects.[1] Accordingly, there is a need to identify novel drugs with tocolytic characteristics, such as calcium agonists, potassium channel openers and other vasodilators.[2] It has been shown that even otomolar concentrations of nicardipine inhibit spontaneous rhythmical activity of the isolated uterus.[3] Nitric oxide (NO) is involved in numerous physiological processes and pathological conditions, and it mediates the relaxing effect of protamine sulphate and other smooth muscle vasodilators.[4] In cells, NO is created under the influence of NO-synthesis.[5] High doses of L-arginine increase blood flow in the heart, mesenterium, lungs and liver without affecting total peripheral resistance and blood pressure.[6] L-arginine, however, can cause significant hypotension in normotensive rats pretreated with physostigmin.[7] Previous reports showed increased NO synthesis during normal pregnancy in animals. In humans, lack of NO causes vasoconstriction and pre-eclampsia. NO is characterised by extreme reactivity to intracellular enzymes and has been shown to affect activity of guanylate cyclase (GC). Reaction between NO and GC can be inhibited with methylene blue.[8]

Pentoxyphylline is a methyl xanthine derivative used to treat peripheral vascular diseases. Potential indications for this drug, as well as its mechanism of action at the molecular level, are being intensively studied.[9]

In previous studies, we showed that the endothelium plays a significant role in the relaxing mechanism of pentoxyphylline in isolated rat mesenteric arteries. In the present study, we examined the effects of increasing pentoxyphylline concentrations on spontaneous rhythmical activity of isolated uteri and on calcium chloride-caused activity in the uterus. To elucidate the mechanism of action of pentoxyphylline in uterine smooth muscle, we studied the drug's effects in the presence of L-arginine (an NO precursor), glibenclamide (a potassium channel antagonist) and methylene blue (a GC inhibitor).

MATERIALS AND METHODS

All protocols for handling rats were approved by the local Ethical Committee for Animal Experiments, which strictly follows international regulations. Isolated uteri of virgin Wistar rats (200–250 g) in oestrus, as determined by daily examination of vaginal lavage, were used in this study. Each uterus was suspended in an isolated organ bath chamber (Ugo Basile) containing De Jalon's solution (NaCl 9.0 g/l, KCl 0.42 g/l, $NaHCO_3$ 0.5 g/l, $CaCl_2$ 0.06 g/l, glucose 0.5 g/l) and aerated with 95% O_2 and 5% CO_2. The temperature was maintained at 37°C. Isometric contractions were recorded using an isometric force transducer (Ugo Basile). The uteri, which were either spontaneously active or induced with 6 mM Ca^{2+}, were allowed to equilibrate at 1 g of tension before experimental drugs were added. After establishing stable spontaneous contractions (approx. 20 min), uteri were treated with increasing concentrations of pentoxyphylline (4.2 mM, 12.8 mM, 29.9 mM, 64.1 mM, 106.9 mM and 192.3 mM) until total cessation of contractions. To explore the mechanism of action of pentoxyphylline on the uterus smooth muscle, we studied its effects in the presence of L-arginine (0.3 µmol), glibenclamide (2×10^{-6} mol/l) and methylene blue (0.9×10^{-6} mol/l), which were added to De Jalon's solution 10 min before pentoxyphylline.

The effects of these treatments on uterine contractions were calculated as the percentage of control contractions in untreated uteri. All data are expressed as the mean ± SEM. Differences between groups were tested by two-way ANOVA with treatment and dose as factors. Differences between groups were considered statistically significant when $p < 0.05$.

Pentoxyphylline, methylene blue, L-arginine and glibenclamide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Salts for De Jalon's solution were obtained from ZORKA Pharma (Sabac, Serbia) and Merck (Darmstadt, Germany). All drugs were dissolved in distilled water except for glibenclamide, which was dissolved in polyethylene glycol.

RESULTS

Effect of pentoxyphylline on spontaneous rhythmical activity and Ca^{2+}-induced contractions of isolated rat uterus

Increasing pentoxyphylline concentrations (4.2 mM, 12.8 mM, 29.9 mM, 64.1 mM, 106.9 mM and 192.3 mM) resulted in concentration-dependent inhibition of spontaneous rhythmical activity and calcium-induced contractions of the isolated rat uterus. The degree of pentoxyphylline inhibitory effect depended on activation type. Pentoxyphylline exhibited a stronger relaxing effect on calcium-induced uterus contractility. For instance, pentoxyphylline concentration of 106.9 mM, which completely inhibited calcium-induced contractions (98.08%), only partially inhibited spontaneous rhythmical activity of the isolated rat uterus (52.94%). Total inhibition of calcium-induced contractions was achieved with lower concentrations of pentoxyphylline (64.1 mM). In the case of calcium-induced contractions, pentoxyphylline caused time-dependent contraction inhibition; increasing pentoxyphylline concentrations resulted in increased duration of the calcium-induced contractility (Figure 1A, B and C).

Effect of pentoxyphylline on spontaneous rhythmical activity of the isolated rat uterus in the presence of glibenclamide and L-arginine

In this series of experiments, we showed that the presence of glibenclamide (2×10^{-6} mol/l) stimulates the relaxant effect of increasing concentrations of pentoxyphylline on the spontaneous rhythmical activity of the isolated rat uterus. In control experiments without glibenclamide,

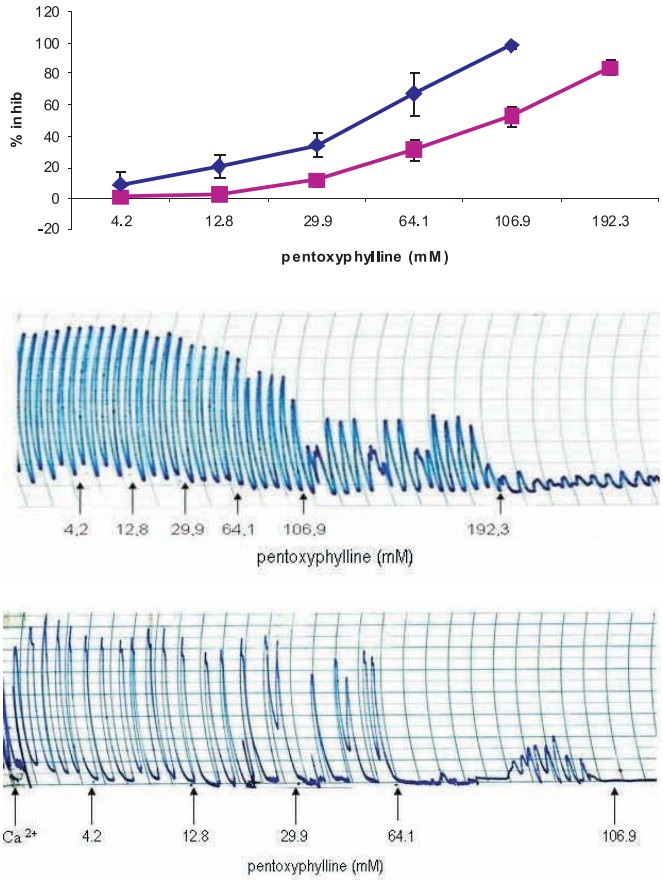


Figure 1.
(A) Concentration–response curves for pentoxyphylline relaxation on the spontaneous rhythmic contractions (closed square ■) and on the contractions provoked by Ca^{2+} (closed diamond ♦) of the isolated rat uterus. The amplitude of contractions just before addition of pentoxyphylline was taken as 100%. The data points represent mean values and the vertical lines indicate the S.E.M. (n = 8–12).
(B) A representative original trace of spontaneous uterine contractions induced by pentoxyphylline at various concentrations (4.2 mM, 12.8 mM, 29.9 mM, 64.1 mM, 106.9 mM and 192.3 mM).
(C) A representative original trace of Ca^{2+}-induced contractions of the rat uterus treated with pentoxyphylline (4.2 mM, 12.8 mM, 29.9 mM, 64.1 mM and 106.9 mM).

pentoxyphylline caused significant inhibition of spontaneous rhythmical activity at a concentration of 192.3 mM. On the other hand, in most experiments a pentoxyphylline concentration of 66.3 mM was sufficient for total inhibition of spontaneous rhythmical contractions in the presence of glibenclamide (Figure 2A and B).

We also studied the effects of increasing concentrations of pentoxyphylline on spontaneous rhythmical activity of isolated the rat uterus and calcium-induced contractions in the presence of L-arginine (0.3 μmol). In the presence of L-arginine, pentoxyphylline did not change its inhibitory effect on spontaneous rhythmical activity or calcium-induced activity.

Influence of pentoxyphylline on spontaneous rhythmical activity of the isolated rat uterus in the presence of methylene blue

In these experiments we studied the effects of increasing concentrations of pentoxyphylline on spontaneous rhythmical activity of the isolated rat uterus in the presence of methylene blue (0.9×10^{-6} mol/l). Methylene blue antagonised the relaxing effect of pentoxyphylline on spontaneous rhythmical activity of the isolated rat uterus. For example, in the presence of methylene blue, even the highest concentration of pentoxyphylline, did not cause complete inhibition of uterine contractions (Figure 3A and B). Interestingly, the addition of pentoxyphylline inhibited uterine contractions, however this inhibition quickly disappeared, and contractions returned to control levels (Figure 3B.).

DISCUSSION

In this study, we examined the effects of increasing concentration of pentoxyphylline on the spontaneous rhythmical activity and Ca^{2+}-induced contractions of the isolated rat uterus. Pentoxyphylline induced concentration-dependent inhibition of spontaneous rhythmic uterine activity and uterine activity caused by calcium. We showed that the degree of inhibition effect by pentoxyphylline depends on the type of muscle contraction activation, and that it is sig-

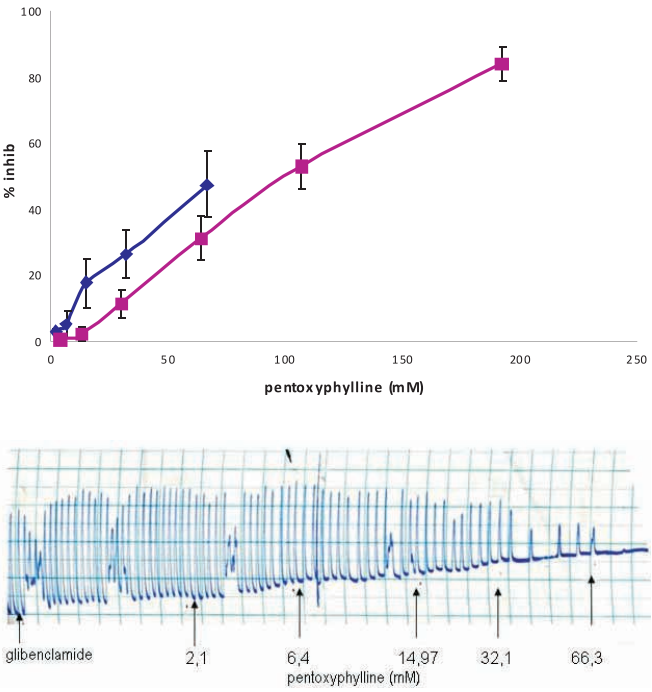
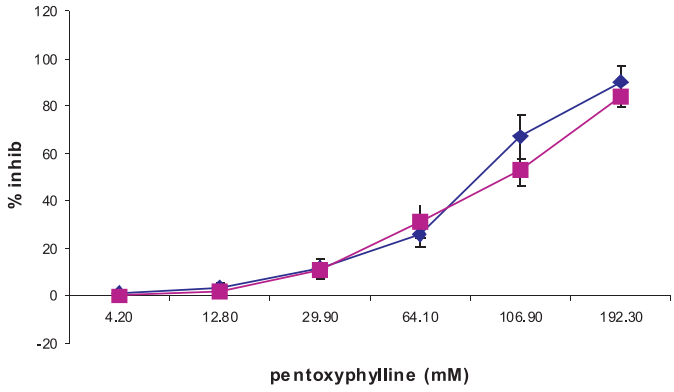


Figure 2.
(A) Effect of pentoxyphylline (4,2 mM, 12,8 mM, 29,9 mM, 64,1 mM, 106,9 mM and 192,3 mM) on the spontaneous rhythmic contractions of rat uteri in the presence (closed diamond ♦) and absence of glibenclamide (2×10^{-6} mol/l) (■ closed square). Contractile activity was expressed as the relative ratio between mean height peak of untreated control and treated uteri. Data are expressed as the mean ± s.e.mean ($n = 8^{-12}$). Pretreatment with glibenclamide significantly increased the relaxing effect of pentoxyphylline ($p < 0.0001$).
(B) A representative original trace showing the effect of (2.1 mM, 6.4 mM, 14.9 mM, 32 mM and 66,3 mM) on the spontaneous rhythmic contractions of the rat uterus, in the presence of glibenclamide.



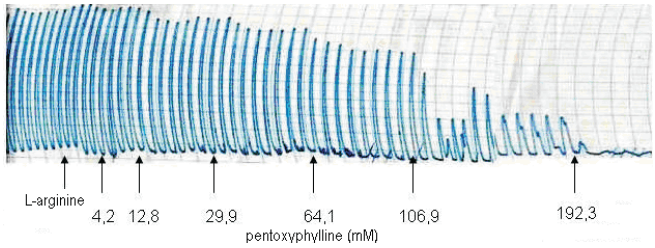


Figure 3.
Effect of pentoxyphylline (4,2 mM, 12,8 mM, 29,9 mM, 64,1 mM, 106,9 mM and 192,3 mM) on the spontaneous rhythmic contractions of rat uteri with (closed diamond ♦) or without L-arginine (2×10^{-6} mol/l) (closed square ■). Contractile activity was expressed as the relative ratio between mean height peak of untreated control and treated uteri. Data are expressed as the mean ± s.e.mean. ($n = 8^{-12}$). (B) A representative original trace showing the effect of pentoxyphylline (4.2 mM, 12.8 mM, 29.9 mM, 64.1 mM, 106.9 and 192.3 mM) on the spontaneous rhythmic contractions of the rat uterus in the presence of L-arginine.

nificantly stronger in spontaneous contractions caused by calcium. These results are consistent with pentoxyphylline being a possible drug that could be used for prevention of miscarriages and premature births.

Apart from finding that pentoxyphylline decreases uterine contractility in a concentration-dependent manner, we also noticed that the degree of the inhibitory effect depended on muscle contraction activation type. That is, pentoxyphylline exhibited differential effects spontaneous rhythmical and spontaneous calcium chloride-induced. The same applied concentrations of pentoxyphylline potently inhibited spontaneous rhythmical activity of the uterus. On the other hand, complete inhibition of rhythmical activity caused by calcium-chloride was achieved with lower pentoxyphylline concentrations.

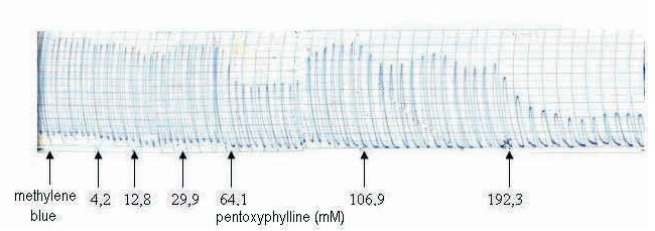


Figure 4.
A representative original trace showing the effect of pentoxyphylline (4.2 mM, 12.8 mM, 29.9 mM, 64.1 mM, 106.9 and 192.3 mM) on the spontaneous rhythmic contractions of the rat uterus in the presence of methylene blue.

Our results showing that the degree of the pentoxyphylline inhibitory effect depends on muscle activation type are consistent with previous reports showing calcium antagonism of uterine smooth muscle contraction. For example, nitrendipine potently inhibited contractions of the isolated rat uterus induced by electrical stimulation, while showing weaker inhibitory effects on spontaneous rhythmical activity and acetylcholine-induced activity, and the weakest effect was observed on oxytocin-induced activities.[3,10]

Spontaneous rhythmical muscle activity is achieved by calcium influx into cells from extracellular spaces through calcium channels located in the cellular membrane. Consequently, spontaneous rhythmical activity depends primarily on calcium concentrations outside the cell. Other types of muscle activation (e.g., induced by acetylcholine or oxytocin) depend on intracellular calcium or calcium that enters the cell by activation of muscarine and oxytocin receptors.[10]

Our results show that pentoxyphylline had a weaker relaxing effect on spontaneous rhythmical activity than on muscle activity in the presence of exogenous extracellular calcium. This result suggests that spontaneous rhythmical activity of muscles could depend on the calcium concentration present in the cell to a greater extent than previously thought.

L-arginine is a precursor of NO and, as such, it can raise the NO concentration, leading to smooth muscle cell relaxation. In human and animal models unable to produce NO due to endothelium dysfunction, L-arginine restores endothelium-dependent vasodilatation. However, in our experiments we found that L-arginine did not antagonise the relaxing effect of pentoxyphylline on the isolated rat uterus. These results suggest that pentoxyphylline achieves its relaxing effect on uterine smooth muscle regardless of the presence of nitric oxide.

In contrast with our findings, in experiments involving renal and mesenteric arteries taken from normotensive and hypertensive rats, it was found that L-arginine antagonised relaxation caused by sodium nitroprusside.[11] The authors proposed a possible explanation of this phenomenon based on the ability of sodium nitroprusside to achieve its action through peroxynitrate and not through S-nitrosothiole.

In our experiments, we found that the relaxing effect of pentoxyphylline was enhanced by the presence of glibenclamide, a selective blocker of K_{ATP} channels. Complete inhibition of contractions in the presence of glibenclamide was achieved using lower pentoxyphylline concentrations.

Potassium channels are present in numerous smooth muscles of the uterus.[12] Recent results show that some known potent vasodilators, such as sodium nitroprusside (an NO donor) and minoxidil, act in part by opening of potassium channels.[13] One of the most studied types of potassium channels, which is dominant in smooth muscles of the uterus, is a large calcium-dependent potassium channel, BK_{ca} or maxi K. This potassium channel is important during gestation and, in particular, during delivery, because its inhibition leads to increased intracellular calcium

levels necessary for birth contractions.[14] ATP-dependent potassium channels (K_{ATP}) also play an important role in uterine smooth muscle physiology; these channels form the connection between the metabolic state of the cell and cell excitation (i.e., contractility).[15]

Changes in the expression and activity of potassium channels are very important for uterus contractility control.[2] For example, H_2O_2 induces concentration-dependent relaxation of the isolated rat uterus via voltage-dependent potassium channels.[16] Based on these findings, studying potassium channel modulators and their effect on NO in uterine tissue is important to better understand uterine physiology and pathophysiology and to help identify new therapeutic concepts in the treatment of uterus contractility disturbances.[2,3] Together with detailed information about potassium channels, there is a pronounced pharmaceutical interest in the synthesis and development of selective potassium channel modulators, as well as re-evaluation of the spectrum of choice of existing drugs and substances influencing permeability of smooth muscle cells membranes for potassium ions (especially vasodilators with so-called direct effects, including monoxidil and diazoxide).

It has been shown that glibenclamide leads to inhibition of contractions of arterial muscle elements, probably by inter-reacting with voltage-dependent calcium channels and by blocking.[17] Our results could be interpreted in the same way. It is possible that glibenclamide stimulates the relaxing effect of pentoxyphylline by blocking voltage-dependent calcium channels, thereby decreasing calcium entry into the cell, causing mus

Methylene blue inhibits production of cGMP by preventing interaction of NO with guanylate cyclase. In our experiments, methylene blue decreased the relaxing effect of pentoxyphylline. Not even the highest applied concentration of pentoxyphylline achieved complete discontinuation of contractions. It is interesting that inhibition caused by pentoxyphylline in the presence of methylene blue has a short duration. That is, while pentoxyphylline inhibited uterus contractions, the duration of this inhibition was brief and contractions returned to the values of the control level.

Data showing that methylene blue antagonises the relaxing effect of sodium nitroprusside are consistent with our results.[5] Methylene blue blocks the activation of guanylate cyclases, thereby preventing muscle relaxation. cGMP activates cGMP-dependent protein kinase, which blocks the entrance of calcium ions, activates potassium channels and decreases levels of IP_3, leading to vasodilatation. However, it has also been shown that methylene blue fails to antagonise the relaxing effect of sodium azide and of nitroglycerol.[18]

Out results suggest that NO is not significantly involved in the realisation of pentoxyphylline effects. There are data about the existence of signaling pathways in the cell, including NO-independent cGMP creation, which does not lead to relaxation. It is possible that methylene blue influences these signaling pathways, leading to weaker relaxing effects of pentoxyphylline in interaction with methylene blue.

According to previous reports, pentoxyphylline acts as a phosphodiesterase blocker.[19] By blocking phosphodiesterase, pentoxyphylline directly increases cAMP levels in muscle cells, leading to muscle relaxation. Since methylene blue, a known inhibitor of guanylate cyclase, decreased the inhibitory effect of pentoxyphylline, it is possible that even cGMP is included in muscle relaxation via NO-independent signaling pathways.

CONCLUSIONS

Pentoxyphylline caused concentration-dependent inhibition of spontaneous rhythmical activity and calcium-induced contractions in isolated rat uteri. The magnitude of the relaxing effect of pentoxyphylline depended on the type of uterus smooth muscle activation. As opposed to methylene blue, L-arginine and glibenclamide did not antagonise the relaxing effect of pentoxyphylline on spontaneous rhythmical activity of the isolated rat uteri. These observations suggest a possible mechanism of action of pentoxyphylline in uterine smooth muscle cells. NO is probably not significantly involved in the relaxing effect of pentoxyphylline, because pentoxyphylline, in the presence of L-arginine, did not significantly affect the degree of contraction inhibition. Because methylene blue antagonised the relaxing effect of pentoxyphylline, we conclude that a signalling pathway that involves cGMP and is independent of NO is possibly involved in the mechanism of its effects on uterine smooth muscle. Our results also suggest that pentoxyphylline could be a potential tocolytic drug.

REFERENCES

1. Kalezić I, Rodić V, Kitanović S, Milovanović G, Zgradić I and Milovanović S. The Effects of Ritodrine, on a receptors in smooth uterine muscle and heart atria of rats. Arch Toxicol Kinet Xenobiot Metab 1993; 1:112-118.
2. Novaković R, Milovanović SR, Heinle H, Protić D, and Gojković-Bukarica Lj. The effect of potassium channel opener pinacidil on non-pregnant rat uterus. Basic & Clinical Pharmacology & Toxicology 2007;1742-84.
3. Milovanovic S R, Ognjanović J, Varagic VM, Bosković B. Effect of nicardipine of the isolated rat uterus and other smooth muscles of the rat. Archives Internationales de Pharmacodynamic et de Therapie 1988; 295: 1348-58.
4. Oreščanin-Dušić Z, Milovanović S, Spasić M, Radojičić R, Blagojević D. Effect of protamine sulfate on the isolated mesenteric arteries of normotensive and spontaneously hypertensive rats. Arch Biol Sci 2008;60:163-168.
5. Orescanin Z, Milovanović SR, Spasic DS, Jones DR, Spasić MB. Different responses of mesenteric artery from normotensive and spontaneously hypertensive

rats to nitric oxide and its redox congeners. Polish Pharmacological Reports 2007; 59: 322-329.
6. Van Geldern EM, Heilingers JPC, Saxena PR. Hemodynamic changes and acetylholine-induced hypotensive responses after N-nitro-L-arginine-methyl-ester in rats and cats. Br J Pharmacol 1991;103:1899-1904.
7. Prostran M, Varagic VM, Todorovic Z, Jezdimirovic M. The effects of physostigmine, L-arginine and N-nitro-L-arginine-methyl-ester (L-NAME) on the mean arterial blood pressure of the rat. J Basic Clin Physiol Pharmacol 1994; 5:151-166.
8. Choi JW, Im MW, Pai SH. Nitric oxide production increases during normal pregnancy and decreases in preeclampsia. Ann Clin Lab Sci 2002; 3:257-63.
9. Matson, PL, Yovich, JM, Edirisinghe, WR et al. An argument for the past and continued use of pentoxyphylline in assisted reproductive technology. Hum Reprod 1995 (Suppl): 10; 67–71.
10. Varagić, VM, Milovanović SR, Srkalović, G. The effect of calcium-channel-blocking agents on the various types of smooth muscle activation of the isolated rat uterus. Arch Int Pharmacodyn 1984; 270; 79-87.
11. Oreščanin Z, Milovanović S. Effect of L-arginine on the relaxation caused by sodium nitroprusside on isolated rat renal artery. Acta Physiologica Hungarica 2006; 93:271–283.
12. Gojković-Bukarica L, Kazić T. Differential effects of pinacidil and levcromakalim on the contractions elicited electrically or by noradrenaline in the portal vein of the rabbit. Fundam Clin Pharmacol 1999;3:527-34.
13. Schubert U, Krien U, Wulfusen I. Nitric oxide donor sodium nitropruside dilates rat small arteries by activation of inward rectifier potassium channels. Hypertension 2004; 43:891-96.
14. Khan R N, Matharoo-Ball B, Arulkumaran S, Ashford M LJ. Potassum channels in the human myometrium. Experimental Physiology 2001; 862: 255-64.
15. Morrison JJ, Ashford MLJ, Khan RN, Smith S K. The effects of potassium channel openers on isolated pregnant human myometrium before and after the onset of labor: potential for tocolysis. Ат J Obstet Gynecol 1993;169:1277-85.
16. Appiah I, Milovanović S, Radojicic R, Nikolic-Kokić A, Orescanin-Dusić Z, Slavić M, Trbojević S, Skrbić R, Spasić MB and Blagojević D. Hydrogen peroxide affects contractile activity and anti-oxidant enzymes in rat uterus. British Journal of Pharmacology 2009;158:1932-41.
17. Crosbie AE, Vuylsteke A, Ritchie AJ, Latimer RD, Callingham BA. (): Inhibitory effects of glibenclamide on the contraction of human arterial conduits used in coronary artery bypass surgery. J Pharm Pharmacol 2000;52:333-40.
18. Milovanović SR, Varagić VM, Kovacević V. The effect of nitro compounds on the metabolism of cGMP and the activity of the isolated rat uterus. Iugoslav Physiol Pharmacol. Acta 1985(Suppl. 3); 21:12-16.
19. Calogero, AE, Fishel, S, Hall, J. Correlation between intracellular cAMP content, kinematic parameters and hyperactivation of human spermatozoa after incubation with pentoxyphylline. Hum Reprod 1998;13: 911-15....