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Progesterone Inhibits Human Myometrial Contractions by its Action on Membrane Receptors

Progesteron İnsan Miyometrial Kontraksiyonlarını Membran Reseptörleri Aracılığı İle İnhibe Etmektedir

Remzi Gökdeniz [1], Ali Ovayolu [1], Robert E.Garfield[2]

[1] Inonu University, Medical Faculty, Department of Obstetrics and Gynecology, MALATYA
[2]Department of Obstetrics and Gynecology, Joseph's Hospital, 445 N. 5th Street, Phoenix, AZ 85004

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ABSTRACT

Purpose: The mechanisms for myometrial inhibition are still being investigated. The aim of this article is to examine mechanisms of progesterone (P4) inhibition of uterine contractility.

Methods: Prospective study Tertiary care center at St. Joseph's Hospital and at Maricopa Hospital, Phoenix, AZ and research center in Arizona, USA. During 2010-2011 24 women were given birth by cesarean section. Uterine tissues from women (n=24) at term were suspended in organ chambers and exposed to various agents. Contractility was recorded and compared before and after addition of agents. Tissues were treated with P4 alone, a progestin (R5020) with low affinity to the progesterone membrane receptor (mPR), or a non-sex steroid (cholesterol). Other tissues were pretreated with inhibitors of adenylate cyclase (SQ 22536), phosphodiesterase (rolipram), nitric oxide (NO) synthases (L-NAME) or a nuclear P4 receptor antagonist (mifepristone, MIF), followed by P4. Data were analyzed by ANOVA.

Results: P4 ($P<0.05$) inhibits uterine contractions. R5020 and cholesterol have little $P>0.05$) inhibitory effects. P4 inhibition is not blocked by MIF, SQ, ODQ, rolipram or L-NAME ($P>0.05$).

Conclusions: P4 rapidly inhibits myometrial contractility by nongenomic mechanisms through action on mPR but not via cAMP, cGMP, or NO

Key Words: Cyclic adenosine monophosphate; myometrium; preterm labor; progesterone receptors; uterine contractility

ÖZET

Amaç: Miyometrialinhibisyon mekanizmaları halen araştırılmaktadır. Bu çalışmanın amacı progesteronun (P4) uterin kontraktilitesi üzerine olan inhibisyon mekanizmasını incelemektir.

Yöntem: Prospektif çalışma St. Joseph Hastanesi Üçüncü Basamak Tedavi Hizmetleri Merkezi ve Maricopa Hastanesi, Phoenix; AZ ile Arizona Araştırma Merkezi, ABD' de gerçekleştirildi. 2010-2011 yılları sırasında 24 kadın sezaryan operasyonla doğum yaptı. Doğum esnasında kadınlardan (n=24) alınan uterin dokuları organ banyolarında bekletilerek çeşitli ajanlara maruz bırakıldı. Kontraktilite, ajanların eklenmesinden önce ve sonra kayıt edildi ve karşılaştırıldı. Dokular ya yalnız P4 ile, ya progesteron membran reseptörüne (mPR) düşük afinitesi olan progestin (R5020) ile veya bir non-seks steroidi (kolesterol) ile muamele edildi. Diğer dokular ise adenilat siklaz inhibitörü (SQ22536), fosfodiesteraz inhibitörü (rolipram), nitrik oksit (NO) sentaz inhibitörü (L-NAME) veya nükleer P4 reseptör antagonistine (mifepristone, MIF) maruz bırakıldıktan sonra P4 ile muamele edildi. Veriler ANOVA ile analiz edildi.

Bulgular: Progesteron ($p<0.05$) uterin kontraksiyonunu inhibe etti. R5020 ve kolesterol ise ($p>0.05$) çok az inhibe edici etkiye sahipti. Progesteron inhibisyonu MIF, SQ, ODQ, rolipram veya L-NAME ($p>0.05$) ile bloke edilemedi.

Sonuç: Progesteron miyometrial kontraksiyonu genomik olmayan mekanizmalarla; cAMP, cGMP veya NO ile ilişkili olmadan, sadece mPR aracılığı ile hızlı bir şekilde inhibe etmektedir.

Anahtar Kelimeler: Siklik adenozin monofosfat, miyometrium, erken doğum, progesteron reseptörü, uterin kontraksiyonu.

INTRODUCTION

Progesterone (P4) has long been thought to regulate uterine contractility[1] and cervical function[2-4] and therefore the onset and progression of labor. Several early studies suggest that 17 alpha hydroxyprogesterone corporate (17P), a synthetic corporate ester of the naturally occurring metabolite of progesterone, might be used to treat preterm labor[5-7] An analysis of all the early studies by Keirse[8] indicated support for the concept that 17P treatment might be effective for preterm labor. Studies completed and published in 2003 showed that 17P could be used to reduce the incidence of preterm labor in women with documented histories of preterm labor[9] and P4 could be used in patients at high risk of preterm labor.[10] However, the mechanism by which these progestins reduce the incidence of preterm birth is unclear. Previous studies examining the direct effects of 17P and P4 on uterine contractility have been inconclusive with some reports of inhibition and other studies showing stimulation or no effect[11,19]. Our recent observations indicate that P4, but not 17P, inhibits spontaneous human uterine contractions in vitro by action on membrane receptors[20].

Uterine contraction and relaxation events are regulated by changes in electrical activity by a complicated series of biochemical events which include changes in ion concentrations regulated by secondary messengers[21-22]. Changes in the polarity of the plasma membrane occur as a result of entry and efflux of ions such as calcium (Ca^{2+}), potassium (K^{+}), sodium (Na^{+}) and chloride (Cl^{-}) across the plasma membrane. The direction of movement of these ions is determined by their concentrations inside and outside the myometrial cells as well as the membrane potential. An increase in intracellular calcium triggers a complex chain of events which culminates in uterine contraction. Contractility is driven by Ca-calmodulin dependent myosin light chain kinase activity and modulated by G-protein coupled receptors. Uterine quiescence is partially maintained by the cyclic nucleotide (cyclic adenosine monophosphate, cAMP and cyclic guanosine monophosphate, cGMP) pathways[22,24]. cAMP is a second messenger which promotes uterine quiescence by inhibition of calcium mobilization and stimulation of efflux from the myometial cell[22]. It is produced when ATP is catalyzed by the enzyme adenylyl cyclase and it is metabolized by phosphodiesterases[22]. Similarly, cGMP is produced by guanlylate cyclase and nitric oxide stimulates this pathway in the myometrium[25].

Progesterone regulates myometrial cell function by interacting with specific progesterone receptors (PR) that act through either or both of two general pathways: 1) A genomic pathway mediated by the classic nuclear PRs (nPRs) or 2) a non-genomic pathway moderated by surface membrane-bound PRs (mPRs). For the genomic pathway, progesterone binding activates nPRs to become transcription factors that alter the expression of specific genes[27-29]. In regards to parturition, the genomic progesterone pathway is thought to result in the quiescence of the myometrium by inhibiting the expression or a variety of proteins thought to be necessary for contractility. For the nongenomic pathway progesterone is thought to interact directly with membrane receptors (mPRs) and influence intracellular signal transduction through Ca^{2+} or cAMP[30].

This aim of this study was to determine the mechanisms by which P4 inhibits human myometrial contractility by using agents which bind to various receptors or block pathways involved in excitation or relaxation within the myometrial cells.

MATERIAL and METHODS

Patient recruitment occurred in the Department of Obstetrics and Gynecology at St. Joseph's Hospital and Medical Center, Phoenix, AZ and at Maricopa Hospital, Phoenix, AZ. Ethical committee approval for tissue collection was obtained from the IRB committees of both

hospitals and recruitment was by written, informed consent from women (n=24) undergoing scheduled cesarean section at term gestation (37-42 weeks).

Tissue samples were studied in research center and details of ethics approval—use of animals for this study was approved by the Institutional Animal Care and Use Committee of St. Joseph's Hospital—Protocol #369. Tissue samples were obtained from the lower uterine segment and collected in Hanks Balanced Salt Solution (HBSS). Myometrial strips (~2x2x10 mm) were prepared (8 strips/uterine sample), ligated from both ends with surgical silk, and placed into the organ chambers. One end of the strip was tied to the bottom of the chamber and the other end was fastened, with the aid of a stainless steel rod, to an isometric force transducer (Harvard Apparatus, South Natick, MA). The strips were equilibrated at passive tension of 2g in Krebs-Henseleit solution, bubbled with 5% CO_2 in air (t=37^o C, pH ~7.4) until spontaneous contractile activity stabilized. The strips were exposed to various agents or solvents (controls). Contractile activity was registered, stored and analyzed. Contractility was compared before and after addition of various agents and following a high KCl concentration (60 mM). Tissues were treated with P4 alone (10^{-6} to 10^{-3} M), a progestin with low affinity to P4 membrane receptors (R5020, 10^{-4}-10^{-3} M), or a non-sex steroid (cholesterol, 10^{-4} to 10^{-2} M). Some tissues were pretreated with selective inhibitors of adenylate cyclase (SQ 22536, SQ, 10^{-4} M), guanylate cylase (ODQ, 10^{-4} M), phosphodiesterase (rolipram, 2 X 10^{-5} M), nitric oxide synthesis (L-NAME, 10^{-3}M) or a nuclear receptor P4 antagonist (mifepristone or RU486, MIF, 10^{-4}M), followed by P4. Mean values for area under the contraction curves were calculated from 4 to 8 tissues per treatment. Percent changes in contractility were estimated from measurements made for 60 minutes prior to addition of an agent and for 60 minutes following an agent. Data were analyzed by ANOVA for statistical differences ($P<0.05$).

RESULTS

Previously we have shown that P4 rapidly (within 1 hour) and dose-dependently inhibits spontaneous and KCl-induced contractility with an ED_{50} of less than 10^{-5} M (10μM) (Ruddock et. al., Am. J. Ob/Gyn, in press). In the studies reported here we used various agents to see if we could mimic the P4 inhibitory effects of P4 or could block the P4 inhibition.

Figure 1 and table 1 demonstrates the P4 inhibition of contractility and that the P4 inhibition is not blocked by mifepristone (Mife), a nuclear progesterone receptor antagonist. Treatment of tissues with R5020, a nuclear progesterone agonist, results in a slight and temporary reduction in uterine contractility (Figure 2 and table 1). Similarly cholesterol, a non-sex steroid, fails to have any significant effect ($P>0.05$) on contractility compared to P4 (Figure 3 and table 1) except at high concentrations and after one hour (see figure 3). The inhibitory responses to P4 are not suppressed ($P>0.05$) by SQ, ODQ, L-NAME, or rolipram (Figures 4-6, table 2).

Table 1. Effects of pretreatment with mifepristone (10^{-4} M) on inhibition of contractility produced by P4 (10^{-4} M). Also shown are effects of R5020 (10^{-4} M) versus P4 (10^{-4} M) and cholesterol at various concentrations. n= number of tissues examined. Mean values +/- SEM. Note that comparison of mean values with different superscript letters indicate significant differences (P<0.05).

Treatment Groups	n	% Change AUC
Mifepristone		
Control	10	-4.8 ± 3.7 [a]
Mifepristone	11	-2.1 ± 3.2 [a]
P4	5	-59.5 ± 7.7 [b]
Mif + P4	5	-64.7 ± 12.9 [b]
R5020		
Control	4	-1.7 ± 2.5 [a]
R5020	4	0.4 ± 1.9 [a]
P4	4	-57.7 ± 2.9 [b]
Cholesterol		
Control	4	-5.2 ± 9.3[a]
10^{-2}M	4	-19.3 ± 3.4 [a]
10^{-3}M	4	-8.6 ± 7.8 [a]
10^{-4}M	4	-1.7 ± 4.7 [a]
10^{-5}M	4	-8.3 ± 5.9 [a]
10^{-6}M	4	-14.3 ± 3.4 [a]

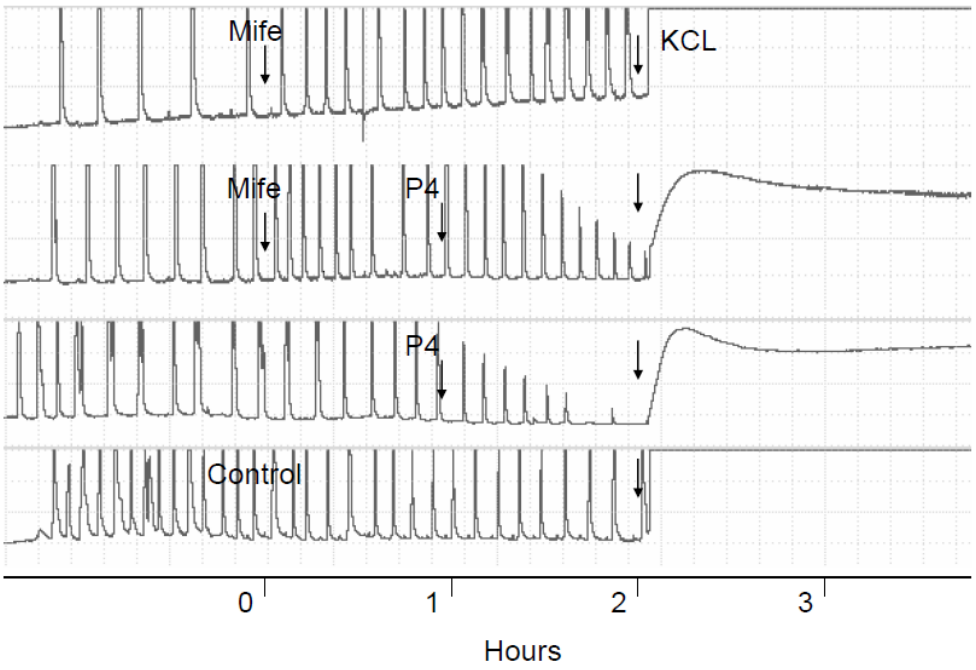

Fig 1. Representative tracings of uterine contractility from four separate tissues showing effects of mifepristone (Mife, 10^{-4} M, top tracing), progesterone (P4, 10^{-4} M, 3rd tracing from top) and pretreatment with Mife followed by P4 (2nd tracing from top) on myometrial contractions. Note that Mife, a nuclear progesterone receptor antagonist, has little effect on contractility and does not inhibit suppression of contractility by P4.

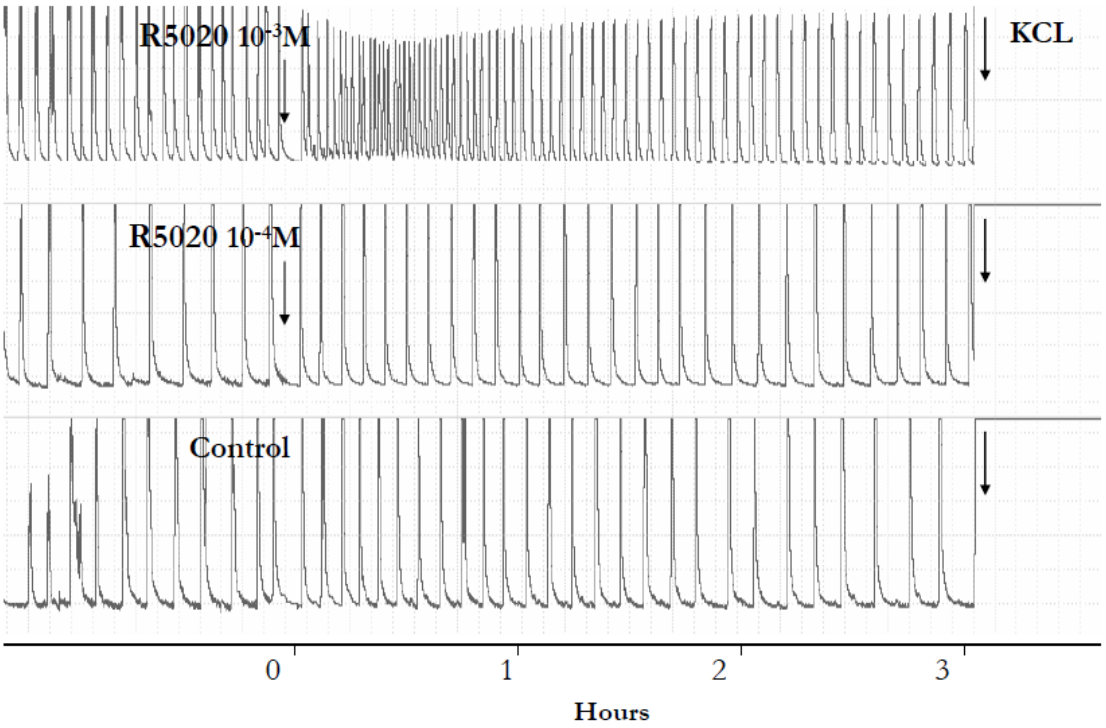

Fig 2. Effects of R5020 (10^{-3} and 10^{-4} M, top two tracings) on myometrial contractility. R5020 is a progestin that binds selectively to nuclear progesterone receptors. R5020 has low affinity to the membrane progesterone receptors and produces transient inhibition at higher concentrations (top trace) as compared to control (bottom trace).

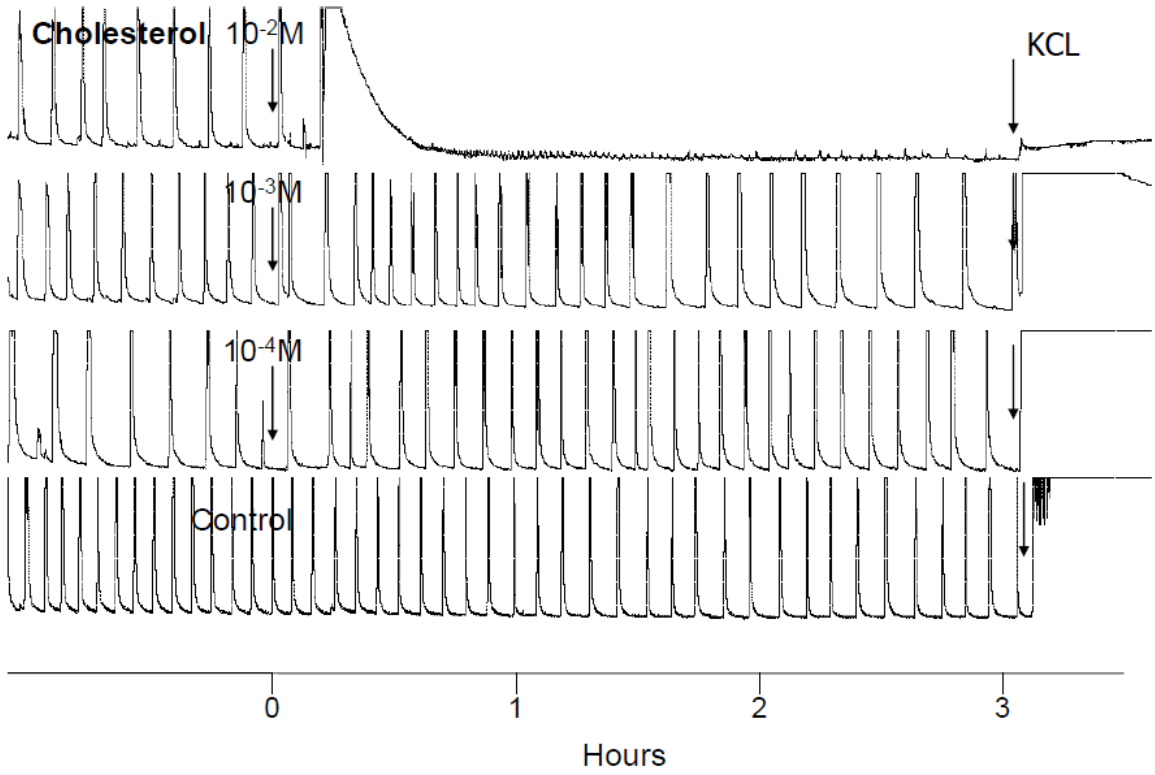

Fig 3. Effects of cholesterol (10^{-2} to 10^{-4} M, top 3 tracings), a non-sex steroid, on myometrial contractility. Note that cholesterol at 10^{-2} M concentration first stimulates contractility and then inhibits (top tracing) as compared to control (vehicle treated, bottom trace). Other concentrations of cholesterol had little effects on contractility.

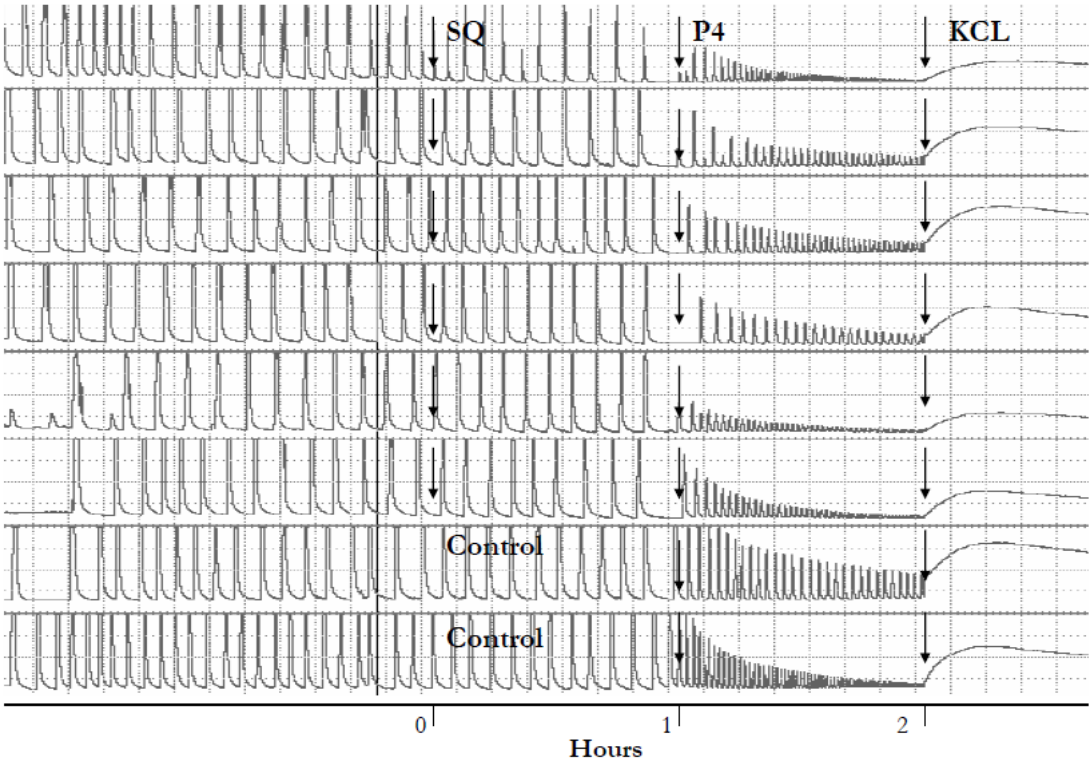

Fig 4. Effects of SQ (SQ 22536, a selective adenylate cyclase inhibitor) on the inhibitory responses to P4. The contractile tracings from one representative experiment are shown. Note that SQ does not block the inhibition of contractility by P4.

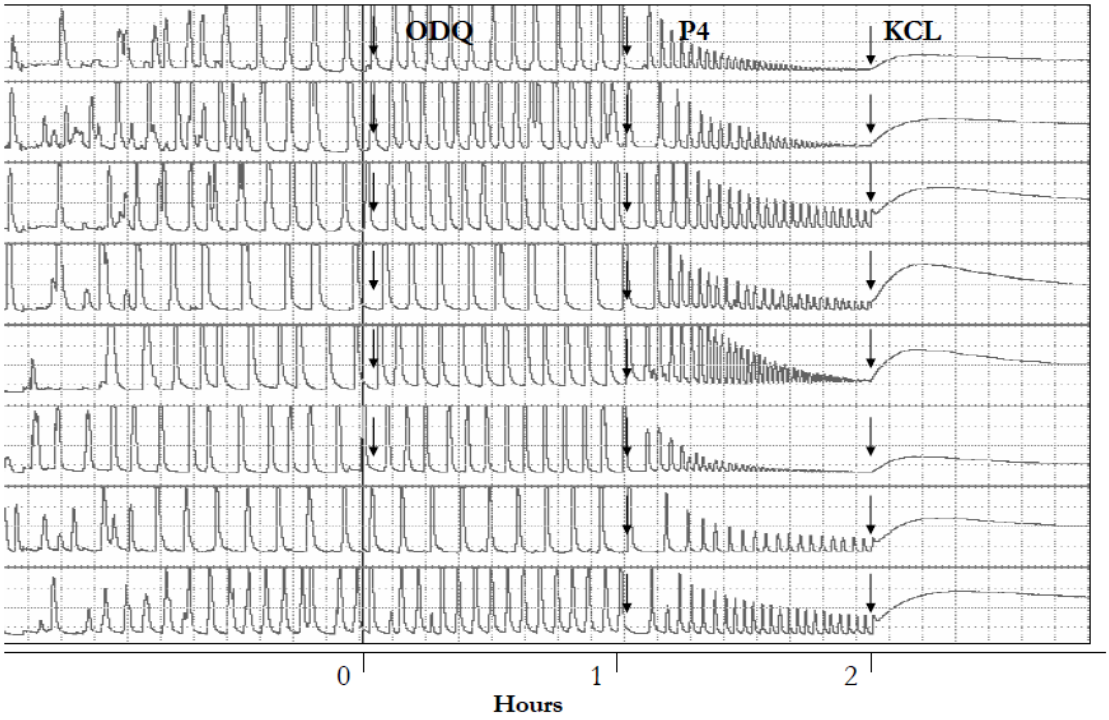

Fig 5. Effects of ODQ (a selective guanylate cyclase inhibitor) on the inhibitory responses to P4. The contractile tracings from one representative experiment are shown. Note that ODQ does not block the inhibitory response to P4.

Table 2. Effects of pretreatment with SQ (10^{-4} M), ODQ (10^{-4} M), L-NAME (10^{-3} M), and rolipram (2×10^{-5} M) on inhibition induced with P4 (10^{-4} M). Data as indicated in table 1.

Treatment Groups	n	% Change AUC
SQ		
Control	4	11.5 ± 12.2 [a]
SQ	6	-17.6 ± 2.4 [b]
P_4	4	-54.9 ± 4.3 [c]
SQ + P_4	6	-62.5 ± 3.0 [c]
ODQ		
Control	4	0.4 ± 2.0 [a]
ODQ	6	20.2 ± 5.9 [a]
P_4	4	-31.3 ± 1.9 [b]
ODQ- P_4	6	-43.9 ± 9.2 [b]
L-NAME		
Control	4	-2.5 ± 0.67 [a]
L-NAME	4	-9.4 ± 1.1 [a]
P_4	4	-59.8 ± 13.6 [b]
L-N+ P_4	5	-59.0 ± 7.6 [b]
Rolipram		
Control	6	-5.3 ± 2.6 [a]
Rolipram	15	-20.2 ± 3.4 [a]
P_4	6	-61.3 ± 17.7 [b]
Roli- P_4	14	-57.0 ± 5.1 [b]

DISCUSSION

Progestins (17P and P4) have been used to inhibit recurrent preterm labor[5,10]. However, their mechanism of action is unclear. Csapo (1 demonstrated that P4 suppresses myometial contractility and others have shown that P4 blocks cervical ripening[2,4]. Thus the premise that P4 regulates the onset and progression of labor is well established. Progestins are generally believed to suppress myometrial contractility through action on nuclear receptors and an inhibition of expression of proteins thought to be important in control of contractility including pathways involved in excitation, propagation and augmentation of contractility[27,29]. Recent studies provide support for the concept that receptors of progestins also exist on the plasma membrane of myometrial cells[30]. These cell surface receptors may too modulate contractility during pregnancy.

We recently demonstrated that P4 rapidly and reversibly inhibits human myometrial contractions in concentrations equivalent to those found locally in the uterus, placenta and blood supply of the uterus (ca. 10^{-5} M). We concluded that P4 directly suppresses myometrial contractility by nongenomic mechanisms through an action on membrane P4 receptors. In this study we provide additional support for the above conclusions.

The P4 inhibitory responses are not blocked by mifepristone, a progesterone antagonist which blocks nuclear membrane receptors. We have demonstrated the lack of effect of mifepristone to block P4 inhibition previously[20] but confirm it in this study. In addition this study demonstrates that similar P4 inhibitory responses are not seen with steroids that do not bind to the P4 membrane receptor (presumably 17P, see reference 20, R5020 or cholesterol). Treatment of tissue with R5020, a progestin with high affinity for the nuclear PRs, results in only minor ($P>0.05$) and transient inhibitory effects on contractility and this is probably due to the low binding capacity of R5020 for the membrane receptors[30]. Similarly cholesterol (10^{-6} to 10^{-2} M, figure 3 and table 1), used in this study as a nonspecific steroid with the possibility of affecting membrane stability, has little effect on contractility except at very high concentrations (10-2 M, figure 3). Our findings are somewhat at odds with another study that used 2.5 times cholesterol levels found in blood[31]. Cholesterol, a precursor for progesterone, is present in the circulation in the 5 mM (5×10^{-3}M) range which is five thousand times the level (1 uM) of circulating progesterone. Our studies suggest that neither guanylate cyclase, cGMP nor nitric oxide are involved in the inhibition of myometrial contractility by P4 as ODQ, a selective blocker of guanylate cyclase, and L-NAME, an inhibitor of nitric oxide synthases, failed to prevent the action of P4. Similarly SQ and rolipram, inhibitors of adenylate cyclase and phosphodiesterase, did not lessen the inhibitory response of P4. We conclude from these results that P4 inhibition is due to a cAMP-independent pathway that at this time remains obscure. Similar inhibitory pathways, both cAMP-independent and –dependent, have been observed in other smooth muscle tissues[32]. Contraction and relaxation of the myometrium are controlled by several pathways which regulate internal $[Ca^{+2}]_i$[22,24]. G proteins are actively involved in intracellular signaling and control of Ca^{+2} and have important roles in regulating myometial contraction/relaxation cycling[22]. Many agents, including B-adrenergic agonists, relaxin and some prostaglandins are thought to relax the myometium by increasing cAMP through stimulation of adenylate cyclase[22]. On the other hand nitric oxide has been shown to stimulate guanylate cyclase and increase the production of cGMP to inhibit contractility of the myometrium[25,26]. Karteris et. al[30] have suggested that P4 binds to membrane receptors of myometrial cells and results in a decline in cAMP levels thereby producing relaxation. It is unlikely that this is the case as a decline in cAMP levels would favor the increase in contractility rather than an inhibition. However,

inhibition of adenylate cyclase with SQ did not change spontaneous activity as seen in this study. Similar findings were obtained with ODQ and L-NAME. Thus these results indicate that neither cAMP, cGMP nor nitric oxide are actively participating in the regulation of contractility in vitro as observed in this study and these agents are not likely to control the inhibition by P4.

The ability of progesterone to suppress myometrial contractility by direct action on receptors located on the surface of muscle cells may be an important mechanism by which progesterone regulates contractility during pregnancy. It is possible that this nongenomic pathway is changed at the end of pregnancy so that P4 inhibition is less and this change also contributes to the onset of labor, as does the genomic pathway[27,29]. Since our study focused on tissues from term, nonlabor patients this would not be revealed by our results. Further studies are required to examine this aspect.

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Details of ethics approval: Use of animals for this study was approved by the Institutional Animal Care and Use Committee of St. Joseph's Hospital—Protocol #369

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Yazışma Adresi / Address for Correspondence:

Dr. Ali Ovayolu

İnönü University Medical Faculty

Department of Obstetrics and Gynecology

MALATYA

e-mail: drovayolu@yahoo.com

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