Studies of cervical ripening in pregnant rats: effects of various treatments

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The exact mechanisms that regulate cervical softening or ripening during pregnancy are not completely understood. The aim of this study was to estimate the effects of various agents on cervical softening during pregnancy in rats. Cervical resistance was examined after treatment with nitric oxide (NO) donors and inhibitors and different hormonal agents. Cervical resistance was significantly reduced ($P < 0.05$) in rats treated with the NO donors: sodium nitroprusside, molsidomine and prostaglandin E₂. However, treatments with the NO synthase (NOS) inhibitors N⁶-nitro-L-arginine methyl ester (L-NAME) and L-N⁶-1-iminoethyl-lysine (L-NIL), or the prostaglandin synthesis inhibitor, indomethacin, significantly increased resistance ($P < 0.05$).

The antiprogesterone, onapristone, reduced cervical resistance and its effects were only partially blocked by the progesterone agonist, promegestone. Relaxin reduced cervical resistance and NOS inhibitors partially blocked the effect of relaxin. These studies demonstrate that NO regulates cervical ripening. Relaxin also softens the cervix and may act by stimulating NO synthesis. Progesterone seems important in the control of cervical ripening, but its role appears complex. NO and prostaglandin pathways may independently control ripening by acting in parallel or synergistically.

Key words: cervical ripening/cervix/nitric oxide/pregnancy/prostaglandins

Introduction

The cervix consists mostly of connective tissue including collagen, elastin and the macromolecular components that make up the extracellular matrix. The extracellular stroma of the cervix includes mainly thick collagen fibre bundles running in all directions and very small amounts of elastin, held together by an amorphous matrix composed principally of proteoglycans, glycoproteins and water (Danforth, 1983; Yu and Leppert, 1991). Collagen is responsible for the tensile strength, elastin imparts elasticity, and the matrix contributes to the integrity of the tissue. Smooth muscle is unevenly distributed throughout the cervix, with a wide variability across species. The cervix of certain animals, e.g. the rat, contains a higher proportion of smooth muscle than that of humans (Harkness and Harkness, 1959). Studies in several species have shown that cervical water content and dry mass increase disproportionately during pregnancy, with the water to dry weight ratio more than doubling around parturition (Harkness and Harkness, 1959; Kleissl et al., 1978; Fitzpartick and Dobson 1979; Woessner and Kokenyesi, 1991).

The role of the cervix shifts between two opposing functions during pregnancy. In order to hold the products of conception inside the uterus, the cervix has to resist tension and remain closed and rigid throughout most of gestation. At term, however, a drastic change in cervical function is required in order to accommodate stretch and delivery (Leppert, 1992). The cervix softens, dilates and effaces at term, a process referred to as cervical ripening.

Endogenous substances thought to control cervical ripening include progesterone (Chwalisz et al., 1991; Chwalisz, 1994; Chwalisz and Garfield, 1994), prostaglandins (PG) (Calder et al., 1977), cytokines (Chwalisz et al., 1994), oestrogens (Cheah et al., 1995) and relaxin (Sherwood, 1988; Sherwood et al., 1990). Uterine contractility is also thought to be involved, at least in the dilatation and effacement phase of ripening. However, the exact role of these factors and the sequence of events that regulates cervical function remain unclear.

Cervical ripening during parturition is thought to depend on extensive alteration within the connective tissue (Mori and Ito, 1991; Leppert, 1998). Cervical ripening has been assessed biochemically by assaying the various connective tissue components, physically by measuring the extensibility of isolated cervical tissue and clinically by visual, digital or ultrasonographic examination (Kleissl et al., 1978; Downing and Sherwood, 1985; Granstrom et al., 1989; Chwalisz, 1994; Cheah et al., 1995; Chwalisz et al., 1997). Recently, the light-induced fluorescence (LIF) of collagen has been used to analyse changes in the cervix during pregnancy (Glassman et al., 1995; Garfield et al., 1998; Shi et al., 1999).

Nitric oxide (NO) has also been implicated in cervical ripening. Studies in rats and guinea pigs (Buhimschi et al., 1996; Chwalisz et al., 1997) as well as humans (Thomson et al., 1997, 1998) suggest that NO effectively softens the cervix. NO may actually represent the central mediator through which the cervical effects of a number of hormonal and inflammatory factors converge. NO is thought to be regulated in part by progesterone (Ali et al., 1997; Chwalisz et al.,...
1997), and cytokines (Nathan, 1992; Moncada and Higgs, 1993). In turn, NO may control the structure and composition of the cervical extracellular matrix through its action on matrix metalloenzymes which, like guanylate cyclase (the target of NO in vascular smooth muscles), are haeme-containing enzymes (Trachtman et al., 1995). The objective in this study was to investigate and to characterize the effect of various hormones and other substances on cervical resistance during pregnancy.

Materials and methods

Animals
Timed-pregnant Sprague–Dawley rats (200–250 g, Charles River Laboratories, Wilmington, MA, USA) were housed separately and allowed free access to food and water. The animals were maintained on a constant 12 h light:12 h dark cycle. The pregnant rats had a gestational period of 22 days, day 1 being the day on which the sperm plug was observed. The animals were anaesthetized with a combination of xylazine (Gemini, Burns Veterinary Supply Inc, Rockville Center, NY, USA) and ketamin HCl (Ketaset; Fort Dodge Laboratories Inc, Fort Dodge, IO, USA) for the intravaginal application of the agents and killed by CO₂ inhalation prior to tissue collection. All procedures were approved by the Animal Care and Use Committee of the University of Texas Medical Branch at Galveston.

Measurement of cervical resistance
The whole cervix, defined as the least vascular tissue with two parallel lumina between the uterine horns and the vagina, was collected. Connective tissue and fat were removed and the cervix was suspended with its longitudinal axis vertically in a 10 ml organ chamber for isometric tension recording (cervimeter). The chamber was filled with physiological Kreb’s solution, bubbled with a mixture of 95% O₂/5% CO₂, and maintained at 37°C. Using hooks inserted through each of the canals, the lower side of the cervix was fixed to the bottom of the organ chamber while the upper was connected to a force transducer which in turn was connected to an online computer. The cervimeter was controlled by a servo device that stretched the isolated cervix incrementally in steps of 0.2 mm at 1 min intervals while the tension was continuously recorded. The resultant length–tension curve had a saw-tooth appearance. As with all actively elastic tissues, each incremental stretch resulted in a sharp rise in tension followed by accommodation and an exponential decrease to a stable tension that was higher than the plateau at the previous length. The slope of the regression line through the linear portion of the length–tension curve was derived and used as an indication of cervical extensibility. The slope of the length–tension curve is related directly to cervical contribution of smooth muscle contraction. There was no resistance to stretch was significant difference between the two groups (unpaired Student’s t-test).

Results

Effect of cervical smooth muscle contractility on cervical resistance
Cervical resistance was measured in isolated cervices from day 14 pregnant rats in calcium-free solutions with EGTA (0.5 mmol/l) and in physiological Kreb’s solution. Calcium-free + EGTA treatment was expected to prevent contraction of smooth muscle in the cervix and to allow measurement of the resistance of the cervical connective tissue without the contribution of smooth muscle contraction. There was no significant difference between cervical resistance measured in normal Krebs’ and that measured in calcium-free solutions (Figure 1).

Effects of NOS inhibitors on cervical resistance
L-NAME (a non-selective NOS inhibitor, 50 mg/day/rat) or L-NIL (a selective iNOS inhibitor, 1 mg/day/rat) were given via osmotic minipumps implanted s.c. Animals were treated for 3, 5 or 9 days before being killed on day 21 of gestation. The resistance to stretch was significantly elevated after 5 days treatment with the NOS inhibitor L-NAME (Figure 2A), but not after 3 or 9 days. The results with L-NIL were similar to those obtained with L-NAME (Figure 2B).
Effects of NO donor and NOS inhibitor on cervical resistance

Rats were treated intravaginally daily with the NO donor, sodium nitroprusside, or the NOS inhibitor, L-NAME, on day 14 of gestation. Sodium nitroprusside (0.3 mg) or L-NAME (0.3 mg) were dissolved in saline and mixed with methylcellulose gel to a final volume of 0.3 ml. Control animals were treated with the vehicle (methylcellulose gel) only. Cervices were collected after 3 days treatment on day 17 of gestation. Sodium nitroprusside significantly reduced cervical resistance compared with control (Figure 3A). Treatment with sodium nitroprusside for shorter periods resulted in variable degrees of softening. Treatment with L-NAME locally did not change cervical resistance significantly, compared with control (Figure 3A).

Effect of PGE2 on cervical resistance

Starting on day 14 of pregnancy, rats were treated with PGE2 gel (0.1 mg/day) intravaginally or gel alone (control), indomethacin (3 mg/day) via osmotic minipumps or vehicle control. Cervices were removed and resistance was measured on day 17 of gestation. The PGE2 gel (Prepidil) decreased cervical resistance (Figure 4A), while indomethacin significantly increased cervical resistance, compared with control (Figure 4B).
Regulation of cervical ripening

Figure 5. The effects of l-N^6^-l-iminoethyl-lysine (l-NIL) and indomethacin on cervical resistance to stretch. Timed pregnant rats were treated with l-NIL (1 mg/day, n = 6), indomethacin (IND, 3 mg/day, n = 5), l-NIL + IND (n = 6) or vehicle (control, saline, n = 5) via osmotic minipumps from days 14–17 of gestation. Data are presented as mean ± SEM. a,b,c Means with common superscripts were not significantly different (one-way analysis of variance followed by Student–Newman–Keuls test).

Figure 6. Effect of relaxin with, and without, nitric oxide synthase (NOS) inhibitors, on cervical resistance. Pregnant rats at day 14 were treated with relaxin (R, 50 µg/day, n = 14), N^ω^-nitro-l-arginine methyl ester (l-NAME) (LN, 50 mg/day, n = 6), l-N^6^-l-iminoethyl-lysine (l-NIL, 1 mg/day, n = 4), relaxin + l-NAME (R + LN, n = 6), relaxin + l-NIL (R + l-NIL, n = 4) and vehicle (control, saline, n = 8) in osmotic minipumps. Cervices were removed and examined on day 17 of gestation. One-way analysis of variance followed by Student–Newman–Keuls test used to compare mean values for statistical differences. a,b,c Means ± SEM with common superscripts were not significantly different (one-way analysis of variance followed by Student–Newman–Keuls test).

Effects of iNOS inhibitor and indomethacin on cervical resistance

Since both NO and prostaglandins may control cervical ripening, rats were treated with NO and prostaglandin inhibitors alone or in combination. Starting on day 14 of pregnancy, the animals were treated with l-NIL (1 mg/day), indomethacin (3 mg/day), indomethacin + l-NIL, or vehicle via osmotic minipumps. The rat cervices were examined on day 17 of gestation. Similar to the results noted above with 3 days treatment, l-NIL had little effect on cervical extensibility while indomethacin significantly increased cervical resistance (Figure 5). Combinations of indomethacin with l-NIL increased stretch resistance to significantly higher values than l-NIL alone (Figure 5).

Effects of relaxin and NOS inhibition on cervical resistance

Timed pregnant rats on day 14 of pregnancy were treated with either relaxin (50 µg/day), l-NAME (50 mg/day) with or without relaxin, l-NIL (1 mg/day) with or without relaxin, or vehicle via osmotic minipumps. Cervices were removed and examined on day 17 of gestation. Relaxin significantly reduced cervical resistance, compared with control (Figure 6). Cervical resistance in rats treated with combinations of relaxin + l-NAME was slightly higher than those treated with relaxin only but this effect did not reach statistical significance. Addition of l-NIL to relaxin, however, significantly increased the resistance compared with relaxin alone.

Effect of progesterone on cervical resistance

Timed pregnant rats on day 17 of pregnancy were treated with onapristone (antiprogestin, 10 mg/rat), promegestone
Figure 7. Effects of the progesterone agonists, promegestone and onapristone on cervical resistance. Pregnant rats were treated with onapristone (O, 10 mg, n = 6), promegestone (P, 2 mg, n = 6), vehicle (control, sesame oil, n = 6) or onapristone + promegestone (O+P, n = 6) administered s.c. on day 17 of gestation. The rats were killed and cervical resistance was measured 6, 12 or 24 h after the start of treatments. At 24 h the animals were bleeding vaginally and had started premature labour. Data are presented as mean ± SEM. a,b,Means with common superscripts were not significantly different (one-way analysis of variance followed by Student–Newman–Keuls test).

(progestin agonist, 2 mg/rat), vehicle (control group, sesame oil) or combination of onapristone + promegestone administered s.c.. The rats were killed and cervical resistance was measured 6, 12 or 24 h after the start of treatment. There was a decline in cervical resistance in control rats consistent with progressive ripening during the 24 h period (Figure 7). In onapristone-treated rats, premature ripening was induced and the animals began to deliver prematurely ~24 h after treatment. The resistance to stretch in the onapristone group was significantly reduced, compared with controls at all time periods. At 12 and 24 h, promegestone increased cervical resistance, compared with the control, but the difference was not statistically significant. Promegestone inhibited premature delivery produced by onapristone and increased cervical resistance, compared with onapristone, but the difference again failed to achieve statistical significance (Figure 7).

Discussion

Previous studies have indicated that cervical ripening, as measured by two independent methods (cervical resistance and light-induced fluorescence of collagen), is a gradual and progressive process occurring during the last half of pregnancy (Garfield et al., 1999; Shi et al., 1999). In this study, it was demonstrated that the physiological process of cervical softening or ripening is controlled by various mechanisms. Some of these mechanisms act independently, while others depend on the same final pathway. Treatment with NOS (L-NAME or L-NIL, Figure 2A,B); and cyclo-oxygenase (COX) (indomethacin, Figure 4B) inhibitors suppressed ripening whereas NO donors (sodium nitroprusside or molsidomine, Figure 3A,B) and prostaglandins (Figure 4A) accelerated it. These data show that NO and PG play important roles in the regulation of cervical ripening.

It has also been shown that, in addition to inducing preterm labour, the antiprogestin onapristone decreased cervical resistance. However, the progestin agonist promegestone completely failed to inhibit ripening caused by onapristone, even though it prevented preterm delivery. Indomethacin also prevented ripening and combinations of indomethacin with NOS inhibitors were more effective than either indomethacin or NOS inhibitors alone in suppressing ripening. Relaxin effectively ripened the cervix and this effect was reversed by NOS inhibition. These data indicate that cervical ripening is controlled by a complex series of steps involving NO, prostaglandins and progesterone.

Recently various authors (Buhimschi et al., 1996; Chwalisz et al., 1997; Thomson et al., 1997,1998; Shi et al., 1998) have suggested that NO may be involved in cervical ripening as one of the local mediators of the inflammatory cascade during term and preterm labour. This study suggests that NOS activity is present in the cervix because competitive inhibitors (L-NIL and L-NAME) block cervical ripening. Inducible NOS (iNOS), a cytokine inducible enzyme, is found in the cervix and is up-regulated when amounts of NO rise in the cervix (Buhimschi et al., 1996). However in our previous studies (Buhimschi et al., 1996; Ali et al., 1997), amounts of NO and NOS were noted to be moderately elevated during the period of decreased cervical resistance shown in the current study (days 12–18 gestation) and then rose significantly on the day of delivery. Nevertheless, Chwalisz et al. have shown that treatment of the cervix with a NO donor (sodium nitroprusside) locally in guinea pigs significantly increases cervical extensibility (Chwalisz et al., 1997), a finding confirmed in rats in the present study. Additionally, our preliminary studies (Buhimschi et al., 1996) and the present results in rats have shown that L-NAME inhibits cervical ripening when given systemically in minipumps, but not locally. Subsequent studies in humans also indicate that NO donors ripen the cervix when applied during the first trimester (Thomson et al., 1997,1998). The effect of NO donors on the human cervix was evident within 3 h whereas 2–3 days were required in guinea pigs (Chwalisz et al., 1997) and rats (this study). The reason for the longer period of treatment required in guinea pigs and rats is not clear. Similarly, NO inhibitors require several days treatment to prevent ripening. This may be because once softening has started due to enzymatic activity it may be difficult to arrest. It is also possible that NO also inhibits cervical contractility and that NO donor treatment results in dilation once softening has occurred. Immediate relaxing effects of NO on cervical smooth muscle have been observed (Ekerhovd et al., 1998). Thus NO could control the slow process of softening as well as the more rapid dilation process. Dilation was not examined in this study, even though it is evident that lack of cervical contractility does not affect cervical softening (Figure 1). Thus, in addition to species variability, the differences in endpoints measured (dilatation versus resistance) and gestational period at which NO was applied (early versus late) between the human and animal studies could have contributed to the difference in response-time. In addition in the present study, both systemic (i.e. osmotic minipumps, drinking water and s.c. treatments) and local (intravaginal) administration was used. This study suggests that it may be possible to manipulate
Regulation of cervical ripening

The cervix is comprised of connective tissue, with collagen being the dominant component (Danforth, 1947; Leppert, 1992). During ripening, remodelling of the collagen is believed to occur and facilitate dilatation during labour. It is often stated that the cervix ripens at term. However, the results of recent studies indicate that softening, as measured by resistance estimates and cervical collagen fluorescence, gradually increases during the second half of pregnancy rather than acutely at term (Garfield et al., 1998; Shi et al., 1999). Other studies in both rats and humans support the concept of a slow ripening process (Harkness and Harkness, 1959; Ekman et al., 1991; Yu and Leppert, 1991). Thus hormonal control must be exerted gradually during pregnancy.

Hormones, especially progesterone (Chwalisz and Garfield, 1994), prostaglandins (Calder et al., 1977; Hollingsworth et al., 1980; Uldbjerg et al., 1981) and relaxin (Downing and Sherwood, 1985), are known to play an important role in control of cervical function. Progesterone seems to exert overall control over cervical ripening, and the antiprogestins induce ripening in all species investigated to date, including humans (Chwalisz and Garfield, 1994). The general consensus is that progesterone inhibits ripening and progesterone withdrawal at term promotes softening (Chwalisz et al., 1991). Progesterone also reduces collagenase activity, leukocytic migration, and suppresses interleukin (IL) synthesis (Jeffery and Koob, 1980; Ito et al., 1994) while antiprogestins promote the opposite (Chwalisz et al., 1991).

It was found that the antiprogestin used in the present study (onapristone) effectively induced softening within 6 h, a significantly shorter time than that required to inhibit or induce cervical softening with NO inhibitors or donors respectively. In contrast, treatment with promegestone failed to add to the softening occurring in control tissues over the 24 h treatment period and did not prevent softening produced by onapristone, even though this combination has been shown to inhibit premature delivery (Garfield et al., 1987). These data suggest that the control of the cervix by progesterone is complex and may involve multiple pathways.

Progesterone inhibits, while antiprogestins stimulate, NO production and NOS synthesis in the cervix (Buhimschi et al., 1996; Chwalisz et al., 1996; Ali et al., 1997). However, the production of NO after onapristone + promegestone or other progestins, does not appear to have been studied. It is possible that with the above combination (antiprogestin + R5020), NO synthesis remains elevated.

Previous studies show that the cervix softens slowly over the last 10 days of gestation (Garfield et al., 1998; Shi et al., 1998). This is the time when circulating plasma progesterone and uterine tissue concentrations are at their highest (Puri and Garfield, 1982). Moreover, concentrations of progesterone are lower postpartum when the cervix reverts to a rigid state (Garfield et al., 1998; Shi et al., 1999). Contrary to previous conclusions concerning progesterone and cervical function (see above), the results of the present study demonstrate that progesterone withdrawal does not control the softening process because the cervix becomes soft well before progesterone concentrations start to decline.

Progesterone is also well known to control uterine contractility in rats during pregnancy (Csapo, 1981). Progesterone withdrawal, which begins at ~day 19 of gestation and nadirs at term, is associated with a number of events thought to be responsible for effective uterine contractility (e.g. increase in gap junctions, ion channels, excitatory receptors) (Puri and Garfield, 1982). Since cervical softening is a slow process with changes appearing over the last 10 days of pregnancy (Shi et al., 1999) and increased uterine contractility and labour is an acute event occurring in the last 12–24 h prior to delivery, these two processes must be regulated by separate and independent mechanisms.

Progesterone withdrawal is also thought to control the influx of leukocytes into the cervix (Chwalisz et al., 1991), and to regulate cytokine synthesis (Ito et al., 1994). The role of cytokines in the recruitment of leukocytes and stimulation of NO and PG production by macrophages and other leukocytes is well-established (Nathan and Xie, 1994). In return, NO can stimulate cytokine and prostaglandin synthesis (Corriveau et al., 1998; Cuthberston et al., 1998). One might conclude that progesterone and its withdrawal control a sequence of increased cytokine synthesis, leukocyte recruitment, and increased production of NO and prostaglandins. However, under these circumstances promegestone would be expected to prevent cervical ripening produced by onapristone if this step is receptor-mediated. This is clearly not the case (Figure 7). These data suggest that control of the cervix by progesterone is more complicated than the sequence suggested above.

Prostaglandins are also thought to contribute to cervical ripening. Prostaglandins are believed to regulate the components of the cervical extracellular matrix in a number of ways. PGF2α increases glycosaminoglycans and the activity of hyaluronic synthases (Murota et al., 1977). Prostaglandins have also been shown to stimulate cytokine synthesis and inhibit protease activity (Denison et al., 1999). PGE2 has been reported to stimulate ‘collagenase activity’ (Goshawaki et al., 1988). However, PGE2 may not actually stimulate collagenase activity (Rath et al., 1987a,b). During cervical ripening, hyaluronic acid may induce the production of interleukin-1 (IL-1). PGE2 seems to dilate small blood vessels in the cervix (Allen et al., 1988) which, in combination with a chemotactic effect (Lange 1983), may increase infiltration of the cervix with leukocytes. Prostaglandins have been used clinically for cervical ripening (Calder et al., 1977; Uldbjerg et al., 1981; Forman et al., 1982). The introduction of prostaglandins, in particular locally administered PGE2, to soften the cervix before labour induction, has been a major advance in obstetrics in the past decade. PGE2-induced cervical ripening is associated with a time-limited enzymatic collagen degradation, increased synthesis of non-collagenous proteins, and a substantial increase in hyaluronic acid concentration (Rath et al., 1990).

The present studies indicate that PGE2 softens the rat cervix (Figure 4A) and indomethacin prevents softening (Figure 4B). These results are similar to the effects of NO donors and NO inhibitors (Figure 3A,B). Treatment of animals with a combination of indomethacin and L-NIL resulted in greater...
suppression of softening than either indomethacin or NO inhibitors alone (Figure 5). These data suggest that there are two independent, and perhaps parallel, pathways that may interact synergistically to control ripening of the cervix. It is also well known that NO stimulates the production of prostaglandins by action on COX enzymes (Salvemini et al., 1993). However, it is unlikely that NO acts solely on COX to produce ripening because indomethacin did not prevent ripening produced by NO donors.

The ripening of the cervix during late pregnancy seems to be the result of an active inflammatory process within the cervix (Junqueira et al., 1980; Liggins, 1981). Inflammatory cells (neutrophils and macrophages) accumulate in the cervix during this process (Junqueira et al., 1980; Liggins, 1981; Chwalisz, 1994). Pro-inflammatory cytokines, e.g. IL-1β, tumour necrosis factor α (TNFα), IL-8, have also been shown to be produced by the cervix and are thought to be involved in softening during pregnancy (Barclay et al., 1993; Chwalisz et al., 1994; Ito et al., 1994). The involvement of NO in inflammatory reactions is well known and its synthesis is regulated by cytokines (Moncada and Higgs, 1993). During the process of ripening, the matrix metalloproteinases (MMP) and metal enzymes e.g. collagenase, could be activated by NO and break down cervical collagen of the cervix thus making it soft. The data in this study are consistent with the concept that cytokines may stimulate both COX and NOS enzymes and thereby regulate cervical ripening. Preliminary reverse transcription-polymerase chain reaction (RT–PCR) studies (M.Ali and R.Garfieid, unpublished) show an increase in MMPase-9 expression following sodium nitroprusside treatment. However, others have not noted changes in MMPase-2 and -9 after NO donors (Ledingham et al., 1999).

There is also abundant evidence that relaxin regulates cervical ripening. Relaxin has two biological effects during pregnancy: it causes uterine quiescence, an effect shared with progesterone, and promotes remodeling of the extracellular matrix of the reproductive tract to accommodate growth and expulsion of the fetus (Bryant-Greenwood, 1982; Sherwood, 1988). Relaxin also regulates cervical function (Sherwood Woessner, F. (eds), 1999). Relaxin can also cause substantial collagen turnover by stimulating collagenase expression as well as modulating collagen synthesis and secretion in human dermal fibroblasts (Unemori et al., 1992). Several recent studies have indicated that relaxin stimulates NO synthesis in a number of tissues (Bani et al., 1995; Bani-Sacchi et al., 1995; Di Bello et al., 1995; Masini et al., 1995,1997).

These results indicate that the action of relaxin on the cervix is mediated by NO but not by prostaglandins, since relaxin significantly lowered the resistance to stretch compared with controls and this effect was inhibited by NO inhibitors (Figure 6). Since the effect of relaxin does not involve prostaglandins (Sherwood et al., 1998), relaxin appears to act specifically on the NO pathway and not the PG pathway regulating cervical softening (see above).

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