Acute Effect of Cocaine Exposure on Pregnant Rat and Human Myometrial Strips and the Role of Endogenous Nitric Oxide Synthesis on this Effect

GEBE SIÇAN VE İNSAN MYOMETRİYAL DOKULARINA KOKAİNİN AKUT ETKİSİNDE ENDOJEN NİTRİK OKSİT SENTEZİNİN ROLÜ

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Summary

The purpose of this study was to assess (in vitro) the role of endogenous nitric oxide synthesis during the acute effect of cocaine exposure on pregnant rat and human myometrial contractile activity Isolated myometrial strips were obtained from day-18 pregnant Albino rats (n=6) and from the lower uterine segment during elective cesarean section of pregnant women (n=7) at term who were not in labor and who had no perinatal risk factors. Myometrial strips were mounted in a standard organ bath for assessment of isometric contraction The response to modulators of nitric oxide synthesis during the acute effect of cocaine was determined. The frequency, duration and the integrated area of contraction expressed relative to control increased acutely after addition of cocaine (10'-10" M) in myometrial strips isolated from pregnant rat (p<0.05). Contraction amplitude, duration and the integrated area, expressed relative to control, increased acutely after addition cocaine (10'-10" M) in myometrial strips isolated from pregnant women (p<0.05). Neither addition of L-arginine (\Q-'-l(r' M), the substrate for nitric oxide synthesis, nor N'-nitro-L-arginine methyl ester (L-NAME) (1Q~'-1(Y' M), an inhibitory of nitric oxide synthase (NOS) led to any specific change in acute effect of cocaine exposure on isolated pregnant rat and human myometrial strips. These results show that endogenous nitric oxide production does not play an important role in acute effect of cocaine exposure on myometrial strips isolated from pregnant rat and human.

Key **Words:** Cocaine, Nitric oxide. Myometrium, Contractions

T Klin J Med Res 1998, 16:36-41

Ozet

Bu çalışmanın amacı gebe sıçan ve insan myometriyal kontraktil aktivitesi üzerine kokainin akut etkisinde endojen nitrik oksit sentezinin rolünün in vitro olarak araştırılmasıdır. İzole myometriyal dokular 18 günlük gebe Albino sıçanlar (n=6) ve doğum eyleminde olmayan ve herhangi bir periuutul riski olmayan terin gebelerin (n=7) uterin alt segmentinden elektif sezaıyen sırasında alındı. Myometriyal dokular Standard organ banyolarına izometrik kontraksiyonlarıu değerlendirilmesi için asıldı ve kokainin akut etkisi üzerinde nitrik oksit sentez modülatörlerine olan cevap belirlendi. Kontrole göre elde edilen kontraksiyon frekans, süre ve eğri altında kalan alan verileri gebe raflardan elde edilen myometriyal dokularda kokain (10~-10~' M) eklenmesinden sonra akut olarak arttı (p<0.05). Kontrole göre elde edilen kontraksiyon amplitüd, süre ve eğri altında kalan alan verileri gebe kadınlardan elde edilen myometriyal dokularda kokain (l()~'-10~' M) eklenmesinden sonra akut olarak arttı (p<0.05). Ne nitrik oksit sentezi substratı olan L-arginin (10'-10" M) ne de nitrik oksit sentaz inhibitörü N^-nitro-L-arginin metil esteri (L-NAME) (İO'^-KY' M) izole sıçan ve insan myometriyal dokularında kokainin akut etkisi üzerinde herhangi spesifik bir etki yapmamıştır. Bu sonuçlar gebe sıçan ve insan myometriyal dokuları üzerinde kokainin akut etkisinde endojen nitrik oksit üretiminin önemli bir rolünün olmadığını göstermektedir.

Anahtar Kelimeler: Kokain, Nitrik oksit, Myometrium, Kontraksiyonlar

T Klin Araştırma 1998, 16:36-41

In the last years, there was a dramatic increase in the use of cocaine in pregnant women (1). Although cocaine use has been associated with several adverse perinatal effects, including an increase in the incidence of small-for-gestational-age infants, pregnancy-induced hypertension, abruptio placentae, and congenital anomalies, perhaps the most significant impact has been an increase in preterm labor and delivery (2). The incidence of preterm delivery among women who use cocaine

Received: Feb. 11, 1998

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has been reported to be 17% to 50% (2-3); however, the mechanisms by which preterm labor occurs in this setting are not well understood. Monga et al. demonstrated that cocaine acutely increases spontaneous contractile activity in myometrium isolated from pregnant rat and human. The mechanism of this effect couldn't be explained completely by inhibiton of catecholamine re-uptake and potentiation of adrenergic pathways (4,5).

Nitric oxide (NO) is a potent smooth muscle relaxant in the blood vessels, the gastrointestinal tract and the respiratory system (6,7). NO is generated from L-arginine by nitric oxide synthase (NOS), which is blocked by the inhibitors of NOS, such as NG-nitro-L-arginine methyl ester (L-NAME), NG-nitro-L-arginine (L-NA) (8,9). NO binds to soluble guanylate cyclase and increases cyclic guanosine monophosphate (cGMP) levels, resulting relaxation of smooth muscle (6,8). Recent evidence has shown that NO has a relaxant (tocolytic) effect on myometrium (10-12). NO is produced within the female genital tract during pregnancy, and a reduction in NO synthesis may be involved in the initiation of parturition (11). Furthermore, the administration of NO donors may be useful by inhibiting uterine contractions in situations where such activity is unwanted, e.g., in preterm labor (13).

The present study was undertaken to investigate the role of endogenous NO synthesis in acute effect of cocaine exposure on myometrial strips isolated from pregnant rat and human.

Materials and Methods

Tissue preparation. Day-18, pregnant Albino rats (n=6) weighting about 200-250 g were cared as mentioned in the guideline of the Cumhuriyet University at Animal Care Center. Animals were killed by cervical subluxation. The uterine horns were rapidly excised and carefully cleaned of surrounding connective tissue and opened longitudinally along the mesenteric border. Fetuses in the late-stage pregnant rats were removed and non-uterine tissues were dissected away and discarded. Pregnant human uterine specimens were obtained from the lower uterine segment during elective cesarean section from term pregnant women (n=7) who were not in labor and who had no perinatal risk factors, in the Department of Obstetrics and Gynecology at Cumhuriyet University Hospital. The average gestation age was 38.2 weeks, with a range 37-40 weeks. The tissues were placed in modified Krebs solution immediately and transferred to the laboratory. The human and rat uterine tissues were cut into longitudinal strips (approximately to 10x2x2 mm length) following the muscle orientation, and were mounted vertically in a 10 ml organ bath containing modified Krebs solution (composition in m mol/L: sodium chloride 125, potassium chloride 2.4, calcium chloride 1.8, magnesium chloride 0.5, sodium bicarbonate 23.9 and glucose 11.0)prcparcd with 95% oxygen and 5% carbon dioxide at 37°C (pH=7.4).

Measurement of contractile activity. The myometrial strips were allowed to equilibrate at 1 g tension for 60-90 minutes before the addition of the experimental drug. The myometrial tension was recorded isometrically with a Grass FT03 force-displacement transducer and registered on a Grass model 79E polygraph (Grass, Quincy, Mass., USA). The recorder was calibrated to a scale that 1 g tension was represented as 1 cm vertical displacement. Paper speed was set at 5mm/min.

Drugs. Chemicals used in the current experiment were cocaine hydrochloride (10-5-10-4), L-arginine (10-5-10-4), L-NAME (10-5-10-4) purchased from Sigma Chemical (St. Louis, Missouri, USA). Drug-containing solutions were dissolved in 0.9% NaCl and added to the bath in volumes of 50 ml at once.

Data analysis. The characteristics of contractions analyzed over 1000-second intervals immediately before and after the addition of drugs included amplitude (grams) of each contraction, frequency (number per 1000 seconds), mean duration (seconds) and integrated area under the contraction curve (representing contractile force over 1000 seconds) measured with a digital plotter. Data are presented as mean±SE and were analyzed by analysis of variance and the Newman-Kuels test with $p<0.05$ was considered significant.

Results

Acute effects of cocaine on spontaneous contractile activity in myometrial strips isolated from pregnant rat and women is evaluated. Cocaine exposure increased spontaneous contractile activity acutely in myometrial strips isolated from pregnant

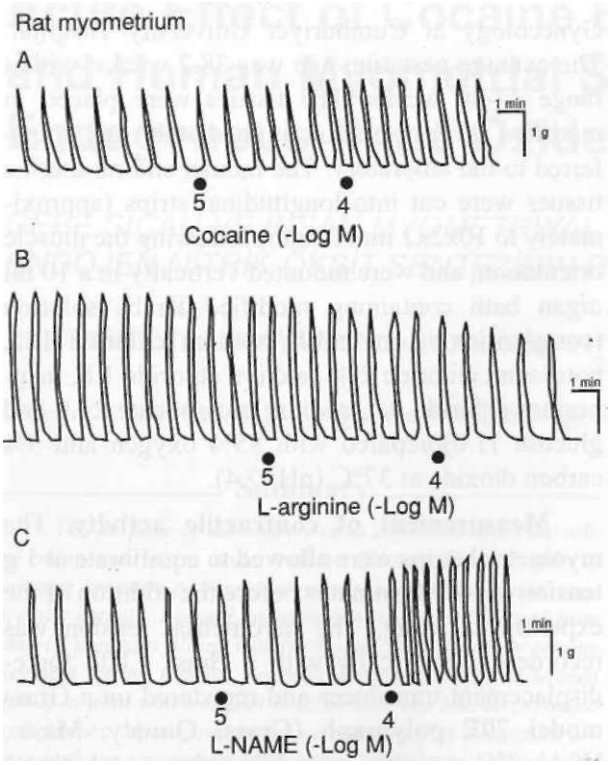


Figure 1. Effects of cocaine (A), L-arginine (B), and L-NAME (C) on spontaneous contractile activity of myometrial strips isolated from pregnant rat.

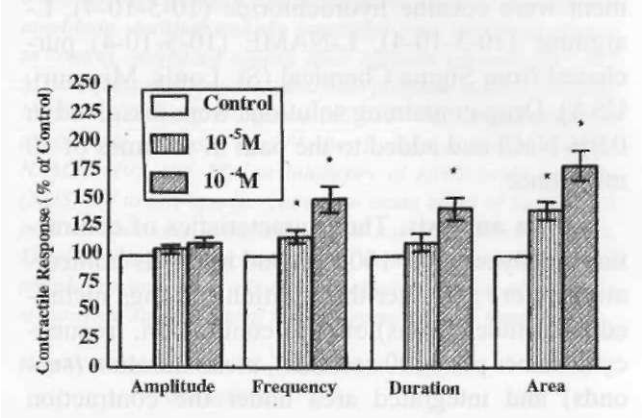


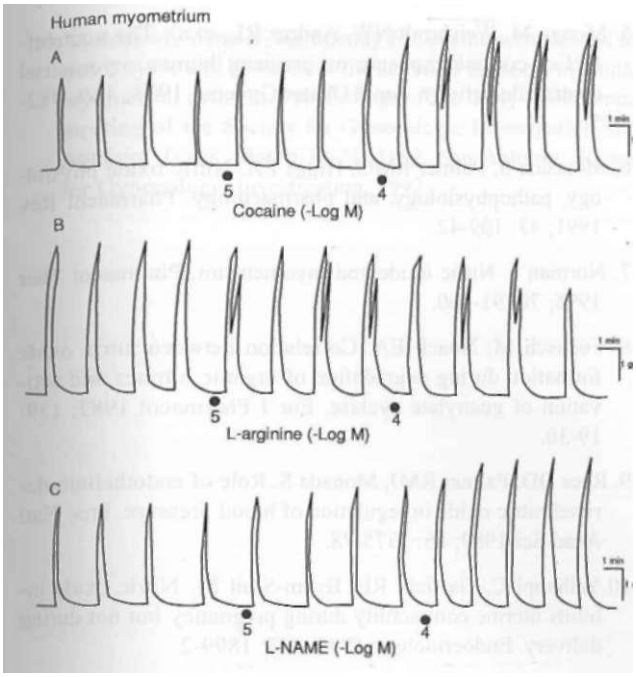
Figure 2. Effect of cocaine on spontaneous contractile activity of myometrial strips isolated from pregnant rat. Data (mean±SE) expressed relative to control (asterisk, $p<0.05$).

onds) measured with a digital plotter. Data are presented as meaniSE and were analyzed by analysis of variance and the Newman-Kuels test with $p<0.05$ was considered significant.

Results

Acute effects of cocaine on spontaneous contractile activity in myometrial strips isolated from pregnant rat and women is evaluated. Cocaine exposure increased spontaneous contractile activity acutely in myometrial strips isolated from pregnant rats, with the greatest effects on the frequency, duration and integrated area of contractions (Figs. 1A, 2). The effects were concentration dependent, reaching statistical significance at the concentration of 10^{-5} and 10^{-4} M. Cocaine had similar effects on spontaneous contractions in myometrial strips isolated from pregnant women (Figs. 3A, 4). Amplitude, duration, and integrated area of spontaneous contractions increased significantly after acute exposure to 10^{-5} and 10^{-4} M of cocaine. A control group was used because of variability in the frequency and amplitude of spontaneous contractions between pregnant rat and women.

. The role of endogenous nitric oxide synthesis in the acute effect of cocaine exposure in myometrial strips isolated from pregnant rat and women is assesed. Figs. IB, 1C, 3B, and 3C show the specificity of the effects of L-arginine and L-NAME on the spontaneous contractions of the myometrial strips isolated from pregnant rat and women. L-arginine decreased uterine contractility in dose-dependent maimer in all myometrial strips, reaching statistical significance at the concentration of 10^{-4} M (Figs. IB, 3B). L-NAME increased uterine contractility in dose-dependent manner in all myometrial strips, reaching statistical significance at the concentration of 10^{-4} M (Figs. 1C, 3C). In pregnant rat and human myometrial strips treated with L-arginine, the decrease in the contractile activity generated was about 27% and 30%, respectively. Both values were significant ($p<0.05$) when compared with the contractile activity (100%) before the addition of L-arginine. In pregnant rat and human myometrial strips treated with L-NAME the increase in the contractile activity generated was about 131% and 138%, respectively. Both values were significant when compared with the contractile activity (100%) before the addition of L-NAME ($p<0.05$). These results indicate that contractility in the pregnant rat and human myometri-



Fi«ure 3. Effects of cocaine (A), L-arginine (B), and L-NAME (C) on spontaneous contractile activity of myometrial strips isolated from pregnant women.

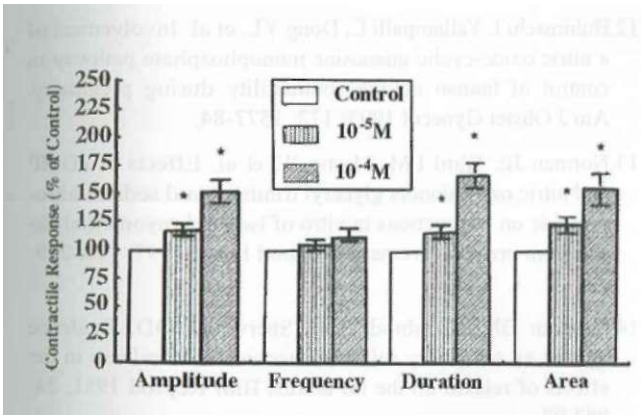


Figure 4. Effect of cocaine on spontaneous contractile activity of myometrial strips isolated from pregnant women. Data (meani-SE) expressed relative to control (asterisk, $p<0.05$).

urn is modified with both the inhibitors and the substrate of nitric oxide.

To assess whether cocaine modified pregnant rat and human myometrial activity through endogenous nitric oxide synthesis, L-arginine (10-4 M), a substrate for NO synthesis, and L-NAME (10-4 M), a inhibitor of NOS, were added to the baths 30 minutes before the addition of cocaine. Acute effect of cocaine exposure in all myometrial strips did not change by the pretrcatmcnt with L-arginine and L-NAME.

Discussion

These findings show that endogenous NO system exists in the pregnant rat and human uterus and that it modulates contractility. This conclusion is based on the following results: (1) myometrial contractile activity is decreased in the response to L-argininc (substrate for nitric oxide synthesis) (2) increased in the presence of L-NAME (inhibitor of nitric oxide synthase). NO is produced by myometrial cells through NOS, and the myometrial NO may be important in maintaining uterine quiescence during pregnancy (14). Preliminary studies suggest that the nitric oxidc-cGMP pathway is present in the human and the rat uterus and the relaxation responsiveness to this pathway is increased during pregnancy and decreased during labor, and the ability of L-arginine to increase cGMP is blocked by L-NAME an mhibitor of nitric oxide synthesis (10-12). The studies examining the changes in the uterine nitric oxide production during different stages of gestation indicate that hi some species, such as human, rat and rabbit (11,12,15) there is an up-regulation with pregnancy and a down-regulation during labor. It is possible that the pregnancy-associated changes in the nitric oxidc-cGMP relaxation pathway may include the changes in nitric oxide production in some species.

Cocaine inhibits catecholamine re-uptake and increases circulating catecholamines. It has been suggested that this action might be the mechanism for cocaine to increase myometrial contractile activity (1,2); however, previous work has demonstrated that there are few functioning adrenergic nerve endings in the term human uterus (16). Furthermore, adrenergic blockage with yohimbine and prazosin did not inhibit the myomclrial response to cocaine in rats (4). These data suggest that facilitation of adrenergic pathways may not be the sole mechanism by which cocaine acutely increases myometrial contractile activity.

The present study was investigated the role of endogenous NO synthesis in acute effect of cocaine exposure on myometrial strips isolated from pregnant rat and human. Pretreatment with L-arginine and L-NAME did not change the acute effect of cocaine in all myometrial strips. These results suggest that endogenous nitric oxide production does not play an important role in acute effect of cocaine exposure on myometrial strips isolated from pregnant rat and human. The increase in preterm labor and delivery among cocaine users could in part be explained by a direct effect of the cocaine on myometrial contractile activity.

Hertelcndy and Molnar (17) and Molnar et al. (18) reported the inhibition of cyclic adenosine 2'-monophosphate generation and stimulation of inositol phosphate generation by cocaine in nonpregnant human cultured myometrial cells. Hurd et al. (19) have demonstrated the down-regulation of (3-adrenergic receptors by cocaine in cultured myometrial cells. These effects were maximal after prolonged exposure (1.5 hours to 4 days). Whether these effects are responsible for the acute response to cocaine observed in our study remains to be elicited.

In summary, this study provides evidence that an endogenous nitric oxide system is present in the pregnant rat and the human myometrium and that it inhibits contractility. But endogenous nitric oxide production does not play an important role in acute effect of cocaine.

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