

Gentamycin inhibition of KCl-induced contractions of myometrium isolated from non-pregnant and pregnant cows

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ABSTRACT: The aim of this study was to investigate the effects of gentamycin on KCl-induced contractions of myometrium isolated from both non-pregnant and pregnant cows. Myometrial strips were isolated from non-pregnant and pregnant cows and suspended in a jacketed organ bath filled with Krebs' solution at 37°C (pH 7.4) continuously bubbled with 95% oxygen and 5% carbon dioxide; isometric contractions were recorded using an isometric force displacement transducer. After manifestation of spontaneous contractions, KCl (60 mM) was applied to the bath and the effects of gentamycin (300 µM, 600 µM) on the amplitude (g) and frequency of KCl-induced contractions were evaluated in 10-minute intervals. Data were statistically analysed using Student's *t*-test and Wilcoxon signed-rank test. Gentamycin inhibited the frequency and amplitude of KCl-induced contractions in a concentration dependent manner. At 300 µM and 600 µM, gentamycin significantly inhibited the amplitude and reduced the frequency of contractions of myometrium isolated from both pregnant and non-pregnant cows. However, an increase in the extracellular Ca⁺ ion concentration virtually reversed this blockade. The results of this *in vitro* study indicate that gentamycin inhibits KCl-induced contractions of myometrium isolated from both non-pregnant and pregnant cows.

Keywords: gentamycin; myometrium; KCl; contraction; cow

It is well known that some antibiotics, including aminoglycosides, depress both skeletal neuromuscular transmission (Liu et al., 2001) and intestinal neuroeffector transmission by a mechanism not related to their antibacterial properties.

It has been shown that the aminoglycoside antibiotic gentamycin inhibits vasoconstriction induced by ET-1 and phorbol myristate acetate in canine cerebrovascular smooth muscle cells (Wickman et al., 2001). Additionally, aminoglycoside antibiotics including gentamycin has been shown to inhibit contractions stimulated with prostaglandin F₂α (PGF₂α, 1 µM) and depolarising concentrations of potassium chloride (KCl, 60 mM) in canine cerebral arteries and in cultured cerebrovascular smooth muscle cells (Gergawy et al., 1998). Gentamycin inhibits a contractile response to KCl in the guinea-pig ileum and rat duodenum by a suggested mechanism of competition with calcium ions (Pimenta et al., 1978; Ozturk et al., 1990). Previous studies including those from our laboratory showed that

the macrolide antibiotics erythromycin (Celik et al., 2001, 2002) and clarythromycin (Celik and Ayar, 2002) had direct inhibitory effects on spontaneous and agonist-induced contractility of myometrium isolated from both pregnant women and rat (Granovsky-Grisaru et al., 1998). More relevantly, aminoglycoside antibiotics including gentamycin were shown to inhibit spontaneous but not oxytocin and prostaglandin E₂ induced contractions of rat myometrium (Paradelis, 1982; Paradelis et al., 1982a,b). Furthermore, despite numerous problems including a tendency to persist in the bovine kidney tissue for several months, the broad-spectrum aminoglycoside gentamycin has been widely used in management of infectious diseases including uterine infections in cattle through both intra-uterine and intra-muscular route (Elezov et al., 1984). Its violative antibiotic residue status in bulk tank milk or in the carcass was studied (Gibbons et al., 1996) but despite its documented inhibitory effect on rat myometrium, to our knowledge, there is no study

in literature investigating the effects of gentamycin on contractility of the cow myometrium.

The objective of this study was to characterize *in vitro* the effect of gentamycin on KCl-induced contractions of bovine uterine smooth muscle isolated from both pregnant and non-pregnant cows.

MATERIAL AND METHODS

Myometrial strips used in this study were obtained from cows slaughtered routinely at the local abattoir. Immediately after slaughter, uterus and ovaries were visually examined to determine any pathologic lesions of the genital tract and myometrial samples were dissected only from cows that were diagnosed to have a healthy genital tract. This was done by later histological confirmation of the tissue collection time inspections. All animals from which tissue samples were used for the experiments were in the follicular phase.

The time from slaughter of cows to collection of uterus and ovaries was about 20 minutes. A single 25×5 mm full-thickness uterine sample from the either horn near the uterine body was removed and rapidly transferred (the average transportation time was about 15 min) from the abattoir to the laboratory by immediately placing it in a flask containing Krebs' solution.

Experiments were commenced within 30 to 120 min of removal of the tissue sample. The tissue samples remained viable for 6 to 12 h of the experiment in these conditions, as judged from stability of the amplitude of spontaneous and $\text{PGF}_2\alpha$ -induced contractions.

Small strips (15 mm in lengths and 5 mm in width, relaxed) consisting of the longitudinal fibre layer of the myometrium were cut from the uterine samples. Only one strip from each animal was used for the same experimental protocol; duplicate samples were used for different parts of the experimental protocol. So, different strips from the same animal were not used to repeat identical procedures.

Strips were immediately placed in a jacketed organ bath containing 20 ml of Krebs solution (containing per litre NaCl 6.9 g; KCl 0.35 g; $\text{MgCl}_2 \times 6\text{H}_2\text{O}$ 0.24 g; NaHCO_3 1.99 g; KH_2PO_4 0.16 g; Dextrose 0.99 g and $\text{CaCl}_2 \times 2\text{H}_2\text{O}$ 0.368 g) at 37°C continuously bubbled with 95% oxygen and 5% carbon dioxide. To measure isometric tension, the distal end of the muscle strips was tied to a fixed glass hook and the proximal end to an isometric force displacement trans-

ducer (Harvard Apparatus Limited, Kent, England). The contractile activities of strips were recorded using a Harvard Universal Oscillograph (Harvard Apparatus Limited, Kent, England).

The myometrial strips were initially placed under 2.0 g of resting tension and a 90-min equilibration period was allowed before the start of each experiment. In previous studies on mare myometrium, 2 g of tension was determined to be optimal (Liu et al., 1998; Crankshaw, 2001). The criteria for identification of contractions were upward going peaks from the baseline at 0.2 grams. Most of the strips developed spontaneous contractions within 30 to 90 min and strips with no regular spontaneous activity in this period were removed.

The following experimental protocol was used: following the stabilization of spontaneous contractile activity in a 90-min equilibration period, 60 mM KCl was added to the tissue bath and contractions were recorded for 10 min, this served as control. Subsequently cumulative doses of gentamycin sulphate were added over a concentration range of 300–600 μM at 10-min intervals, respectively, and the responses were recorded for another 10 minutes. This was performed for all gentamycin concentrations.

Results for amplitude are reported in terms of gram (g) and results for frequency in terms of contractions per 10-min period. The effects of gentamycin sulphate on amplitude (g) and frequency of contractions (number of contractions/10-min period) were analysed for 10-min periods. The mean amplitude and frequency of contractions were compared.

The drug used in the present study was gentamycin sulphate (Gentasol, Eczacibasi, Istanbul, Turkey), and the ingredients of Krebs' solution were in research grade and were obtained from Sigma (Disenhofen, Germany).

All values are expressed as means \pm SEM. Statistical analysis was performed with SPSS Release 9 for Windows (SPSS Inc, Illinois, CA). All the data sets were first tested for normal distribution using the Shapiro-Wilk test for normalcy.

The mean change in amplitude and frequency if uterine contractions occurred before and after drug applications and from the previous dose were tested as appropriate by Student's *t*-test, or Wilcoxon signed-rank test or one- or two-way repeated-measures ANOVAs, followed by Dunnett's *post hoc* analysis. Changes in the rate of uterine contractions were analysed using Friedman's test, followed by

Wilcoxon *post hoc* tests. Statistical significance was assumed at the 5% level.

RESULTS

The tissue samples were left in the organ bath, and after 1 to 1.5 hours spontaneous contractions became established in 16 out of the 22 myometrium samples studied. Three samples without spontaneous contractions and the other three samples with irregular spontaneous contractions during the equilibration period were removed from the study.

In uterine strips from pregnant cows, gentamycin sulphate caused significant inhibition of the KCl-induced contractile activity (Figure 1). The amplitude of KCl-induced contractions was significantly

smaller after cumulative application of 300 and 600 μM concentrations of gentamycin (KCl-induced: 3.48 ± 0.14 g vs. 300 μM Gentamycin: 1.50 ± 0.19 g and 600 μM Gentamycin: 0.22 ± 0.06 g, respectively; $P < 0.001$, $n = 7$). The frequency of KCl-induced contractions of uterine strips from pregnant cows was also significantly inhibited by gentamycin sulphate [(KCl-induced: 12.8 ± 0.7 vs. 300 μM Gentamycin: 4.4 ± 0.6 , $P < 0.01$ and 600 μM gentamycin: 1.8 ± 0.3 , $P < 0.001$, $n = 7$; Figure 2)].

Gentamycin also inhibited the KCl-induced contractions of myometrium isolated from non-pregnant cows. The mean amplitude of KCl-induced contractions was 2.48 ± 0.13 g ($n = 9$) under control conditions and inhibited to 1.27 ± 0.15 g ($P < 0.05$, $n = 9$) and 0.34 ± 0.12 g ($P < 0.001$, $n = 9$) after cumulative application of 300 μM and 600 μM gentamycin,

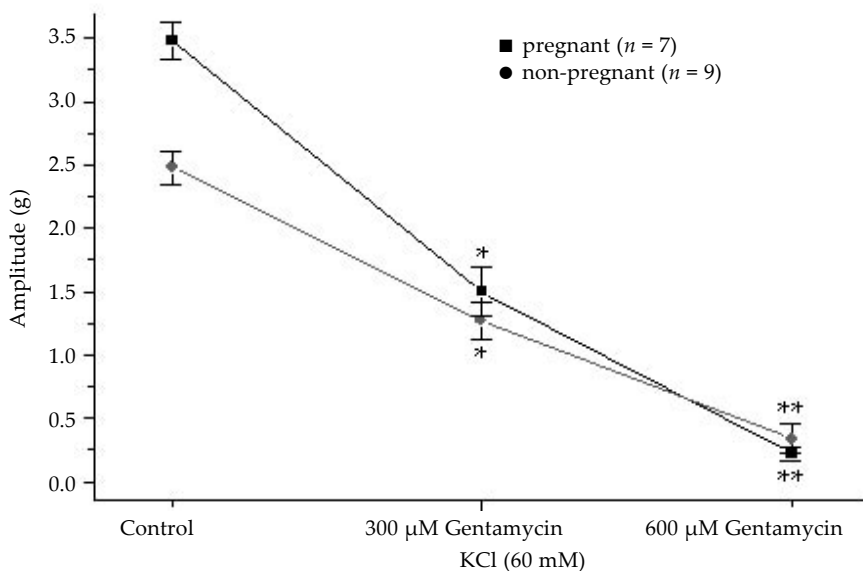


Figure 1. Dose-dependent effects of gentamycin on the amplitude of KCl-induced contractions of cow myometrium. The data are presented as means \pm SEM. * $P < 0.05$ and ** $P < 0.001$ control vs. corresponding dose of gentamycin

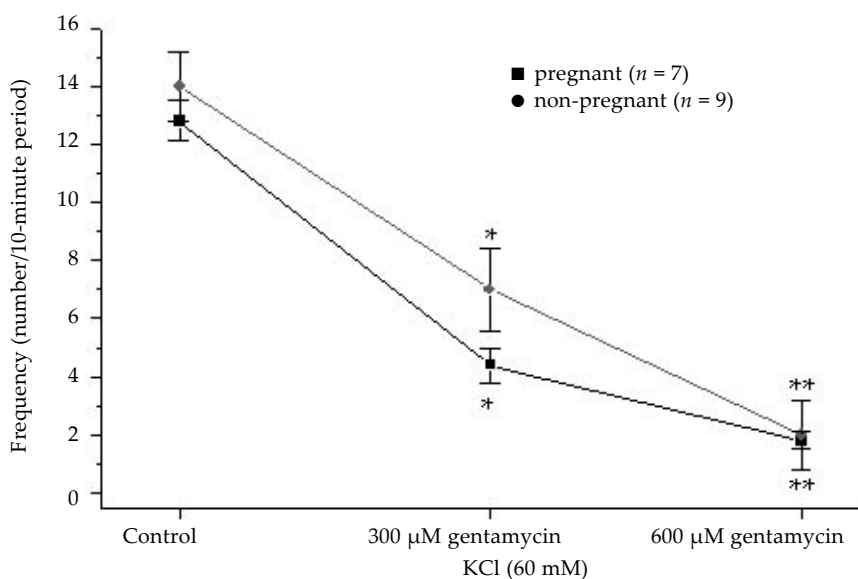


Figure 2. Dose-dependent effects of gentamycin on the frequency of KCl-induced contractions of cow myometrium. The data are presented as means \pm SEM. * $P < 0.05$ and ** $P < 0.001$ control vs. corresponding dose of gentamycin

respectively (Figure 1). Application of gentamycin also caused a significant decrease in the frequency of contractions of non-pregnant cow myometrium [(KCl-induced: 14 ± 1.2 ($n = 9$), 300 μM gentamycin: 7 ± 1.4 ($P < 0.05$, $n = 9$) and 600 μM gentamycin: 2 ± 1.2 ($P < 0.001$, $n = 9$)].

In a subset of experiments ($n = 5$ for each group), the effects of rising extracellular calcium concentration on the gentamycin-induced inhibition of myometrial contractions were tested. For this purpose, at the end of the 10-minute period of the final gentamycin concentration calcium concentration in the Krebs' solution was increased from 2.5 mM to 3.5 mM by adding 1 mM CaCl_2 . When calcium was added, the inhibitory effect of gentamycin sulphate was removed and the normal contractility of the uterus was restored ($n = 5$ for pregnant and non-pregnant myometrial strips after inhibition by 600 μM gentamycin sulphate).

DISCUSSION

It has been clearly demonstrated in the present study that gentamycin sulphate significantly depressed both the amplitude and frequency of KCl-induced contractions of myometrium isolated from pregnant and non-pregnant cows.

In addition to four other aminoglycoside antibiotics, gentamycin was previously shown to inhibit the spontaneous contractility of the isolated rat uterus (Paradelis et al., 1982a,b). But it was found that aminoglycoside antibiotics were not effective on oxytocin and prostaglandin E2 induced contractions of the rat uterus (Paradelis et al., 1982a,b). In the present study involving isolated pregnant and non-pregnant cattle uterus gentamycin inhibited KCl-induced contractions.

The inhibition of calcium channels is a way of controlling the uterus contraction. There are numerous kinds of receptors and ion channels involved in the uterine smooth muscle contraction (Wray, 1993). Though some mechanisms remain to be determined, the molecular mechanism involved in the regulation of labour and myometrial contractions in many species including humans, sheep and cows was substantially elucidated and was reviewed elsewhere (Wray, 1993; Lye et al., 1998; Hirsbrunner et al., 2002).

The control of myometrial contractions is of importance not only to understand and modulate the normal parturition but also to intervene in pre-

term delivery and postpartum involution of the uterus.

Although not many attempts were made to clarify the mechanism by which gentamycin blocks cow myometrial contractions in this study, in agreement with previous studies using isolated myometrial strips (Paradelis et al., 1982a,b) after the establishment of maximum inhibitory effect calcium significantly restored the normal contractility (data not shown). Reversal of gentamycin-induced inhibition of myometrial contractions by extracellular Ca^{2+} (4 mM) provided evidence that the inhibition of membrane calcium channels might mediate this aminoglycoside antibiotic-induced inhibition of myometrial contractions and that the inhibition was due to interaction with the contractile apparatus rather than to the effect on the tissue viability.

Another possible mechanism that may mediate the inhibitory effect of gentamycin on myometrial contractility is inhibition of local prostaglandin synthesis. It was reported in a recently published study that gentamycin significantly inhibited both basal and arachidonic acid or oxytocin-stimulated amniotic prostaglandin E release (Vesce et al., 1999). Furthermore, in an *in vitro* study investigating the mechanism of aminoglycoside-induced ototoxicity gentamycin was found to inhibit the synthesis of prostaglandins by the vascular structures of the inner ear (Escoubet et al., 1985). Because the inhibition was rapid, a possible involvement of an ion channel (in this case Ca^{2+} channels) rather than a metabotropic effect is more plausible.

There was no significant difference between the myometrial strips from pregnant or non-pregnant cows with respect to the inhibition of KCl-induced contractions by gentamycin suggesting the inhibitory effect of gentamycin was due to its interaction with the contractile apparatus not related to the hormonal state of the tissue. We tested the effect of gentamycin on longitudinal layers of the cow myometrium in contrast to the circular layers which were reported to have no cyclic dependent difference in terms of spontaneous contractility.

There is a need for further studies involving electrophysiological (patch clamping) experiments on isolated myometrial cells to clarify the mechanism of gentamycin action on cow uterus. Nevertheless, the results from this *in vitro* study indicated that gentamycin inhibited the contractility of cow myometrium; it also deserves further investigations with regard to possible *in vivo* effects of gentamycin

on uterine activity in postpartum cows when used for intrauterine infections.

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