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NEOMYCIN INHIBITION OF HORMONE-STIMULATED SMOOTH MUSCLE CONTRACTIONS IN MYOMETRIAL TISSUE

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These studies sought to determine the effects of neomycin, a phospholipase C inhibitor, on hormone-stimulated myometrial contractions. For these studies, computer digitalized *in vitro* isometric contraction data were analyzed for changes in contractile activity in response to oxytocin and aluminum fluoride with and without neomycin. Neomycin (1-5 mM) produced dose-related inhibition of oxytocin and aluminum fluoride-stimulated myometrial contractions. This neomycin effect was apparent within 2-3 minutes of addition and was completely reversible, with resolution of its inhibitory effects within 6-8 minutes of washout. This study is the first to demonstrate the functional effect of neomycin inhibition of the phosphatidylinositol signaling pathway in myometrial smooth muscle tissue.

Phasic contractions of smooth muscle tissue, including that present in the female genital tract, occur simultaneously with oscillations of cytosolic calcium [1,2]. Oxytocin-stimulated cytosolic calcium oscillations, as demonstrated by Lynn et al. [3] using cultured uterine myocytes, are consistent with those previously reported to occur in multiple other types of nonexcitable cells in response to various hormones and neurotransmitters [4-6]. Previous reports have confirmed a significant role for the phosphatidylinositol (PI) signaling pathway during the generation and maintenance of cytosolic calcium oscillations [7-9]. Receptor activated stimulation of phosphoinositide-specific phospholipase C (PI-PLC) resulting in increased inositol 1,4,5-trisphosphate (IP_3) production leads to the release of sequestered intracellular calcium; these events then trigger the onset of sustained oscillations of cytosolic calcium. Activation of additional components of the cytosolic calcium oscillation mechanisms, including calcium-induced calcium release (CICR) and facilitation of the influx of extracellular calcium through membrane calcium channels,

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also appear to occur during hormone-stimulated myometrial contractions [11].

Neomycin has previously been reported to inhibit hormone-stimulated IP_3 production and the subsequent mobilization of intracellular calcium in several cell types, including smooth muscle cells [12-15]. To date, however, there are no studies evaluating the functional effect of neomycin on smooth muscle contractile activity. Therefore, the studies described in this report were performed to evaluate the effects of neomycin on phasic myometrial contractions stimulated in response to oxytocin, a hormone agonist previously reported to mediate its effect through activation of the PI signaling pathway [16]. In addition, studies were performed to evaluate the effects of neomycin on myometrial contractions stimulated in response to aluminum fluoride, a direct G-protein agonist that similarly activates the PI signaling pathway [17].

MATERIALS AND METHODS

For these studies, uteri were surgically obtained from proestrus/estrus Sprague-Dawley white rats, rinsed in normal saline, then placed in Earle's buffered saline solution (EBSS) (117 mM NaCl, 1.8 mM $CaCl_2$, 5.3 mM KCl, 0.8 mM $MgSO_4$, 1 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, and 5.6 mM glucose) aerated with 95% O_2 / 5% CO_2. *In vitro* isometric contraction studies were performed as previously reported from our laboratory [11,18]. Oxytocin (0.48 mU/mL) and aluminum fluoride (generated by a combination of 10 μM $AlCl_3$ and 1 mM NaF) were utilized to stimulate a significant increase in *in vitro* myometrial contractile activity; subsequently, neomycin was added to the muscle baths over a concentration range of 1 to 5 mM. Neomycin washout experiments were performed by rapidly rinsing the muscle strips with warmed normal saline twice, then refilling the muscle baths with warmed, pre-aerated EBSS; subsequently, the strips were re-stimulated with oxytocin (0.48 mU/mL).

To quantify the results of these experiments, the isometric contraction data were normalized for tissue cross-section area, and reported as the percent of hormone-stimulated contractile activity as previously reported [11,18]. Statistical analysis was performed using Kruskal-Wallis one way analysis of variance on ranks and the Student-Newman-Keuls multiple comparisons test where appropriate (significance = $p < 0.05$).

RESULTS

As demonstrated in Figure 1, oxytocin, at a concentration of 0.48 mU/mL, produced a marked increase in phasic myometrial contractile activity; subsequently, the addition of neomycin resulted in dose-related inhibition of the myometrial contractions. Utilizing analysis of variance, this neomycin effect was found to be significant (ANOVA on ranks: $H = 30.02$ $p<0.01$), with the effect of neomycin being significantly different from the contractile activity recorded either before the addition of neomycin, or in

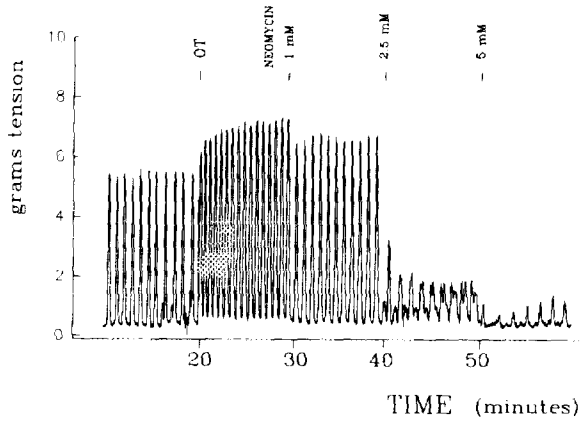

Figure 1.
Cumulative dose response effects of neomycin on myometrial contractile activity. Activity in grams tension generated during the spontaneous contraction period preceding 20 minutes, in response to 0.48 mU/mL oxytocin (OT), and the cumulative additions of neomycin.

myometrial strips treated with an equal volume of vehicle (deionized, distilled water) alone (both $p<0.05$) (Figure 2). As observed in Figure 3, aluminum fluoride-stimulated contractions were also significantly inhibited in response to the same concentrations of neomycin ($H = 32.29$, $p<0.01$).

Figure 4 demonstrates representative data from one of the neomycin washout experiments. As observed, the inhibitory effect of neomycin occurred within 2-3 minutes of

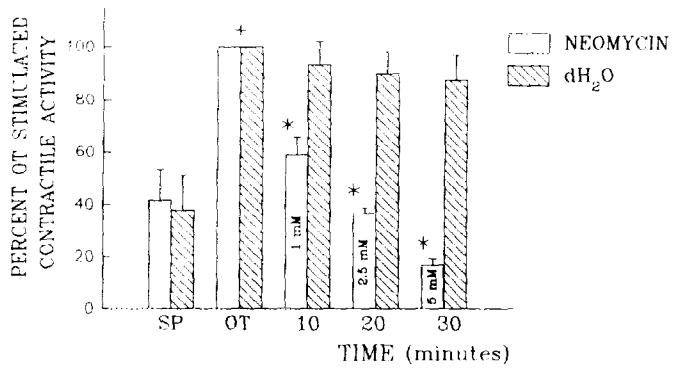

Figure 2.
Quantitative effects of neomycin on oxytocin (OT)-stimulated contractions (as the percent of OT-stimulated activity). Time in minutes after OT stimulation. Each bar = mean ±S.D., N = 4 experiments. (+) = $p < 0.05$ for OT effect compared to spontaneous (SP) contractile activity; (*) = $p < 0.05$ for neomycin-treated strips compared to OT stimulation and vehicle (distilled water) controls.

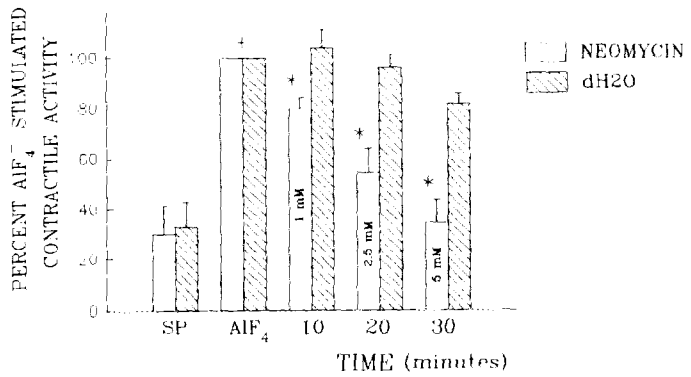

Figure 3.
Quantitative effects of neomycin on aluminum fluoride (AlF_4^-)-stimulated contractions (as the percent of AlF_4^--stimulated activity). Time in minutes after AlF_4^- stimulation. Each bar = mean ±S.D., N = 4 experiments. (+) = $p < 0.05$ for AlF_4^- effect compared to spontaneous (SP) contractile activity; (*) = $p < 0.05$ for neomycin-treated strips compared to AlF_4^- stimulation and vehicle (distilled water) controls.

addition to the muscle bath. Upon washout, the complete resolution of the neomycin effect was similarly rapid (occurring within 6-8 minutes). After washout the neomycin effect was completely reversible; ie. the contractile activity stimulated before the addition of neomycin was comparable to that observed after neomycin washout (Figure 4).

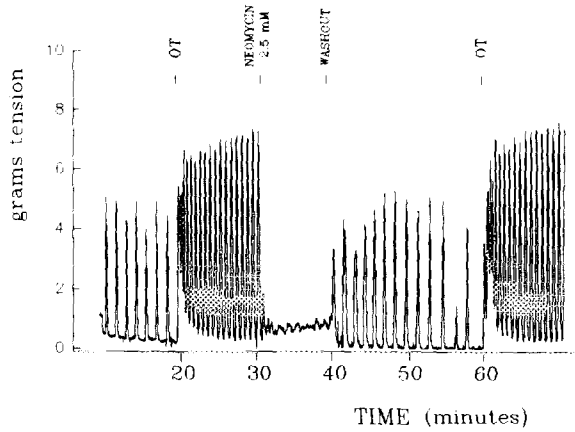

Figure 4.
Representative contraction study confirming the reversibility of the neomycin effect on oxytocin (OT)-stimulated contractions. Activity in grams tension generated during the spontaneous contraction period preceding 20 minutes, in response to OT (0.48 mU/mL), during buffer changes resulting in neomycin washout, and in response to re-stimulation with OT.

DISCUSSION

These studies are the first to report the functional effect of neomycin inhibition of the PI signaling pathway on hormone-stimulated smooth muscle contractions. Consistent with the previously published reports of neomycin inhibition of IP_3 production and intracellular calcium mobilization, these in vitro isometric contraction studies have confirmed that neomycin produces a dose related inhibition of hormone-stimulated phasic myometrial contractions.

In 1986, Swann and Whitaker reported the effects of microinjection of neomycin to a concentration of 10 mM into sea urchin eggs [12]. These investigator observed that neomycin had no effect on calcium release in response to injected IP_3; whereas, it very effectively inhibited the propagation of the wave of calcium release throughout the egg, a phenomena dependent on phospholipase C activation resulting in new IP_3 production. Margolis and coinvestigators, using dissociated rat chromaffin cells, confirmed the utility of neomycin as a membrane permeant inhibitor of the PI signaling pathway [13]. These investigators reported inhibition of bradykinin and histamine induced calcium release in the chromaffin cells; an effect that was apparent within minutes of exposure of the cells to neomycin.

Utilizing rat aortic smooth muscle cells, Kondo et al. demonstrated inhibition of vasopressin stimulated IP_3 generation and the subsequent influx of extracellular calcium after exposure to neomycin [14]. For both of these phenomena, the neomycin effect was dose-related over a concentration of 0 to 5 mM. Also using rat aortic smooth muscle cells, Little and coworkers demonstrated neomycin inhibition of endothelin-1 stimulated IP_3 formation and the mobilization of intracellular calcium [15]. Of note, neither of these smooth muscle studies reported the functional effects of neomycin inhibition of the PI signaling pathway on contractile activity. The present report provides this functional information: neomycin significantly inhibited phasic myometrial contractions generated in response to oxytocin and aluminum fluoride, both known activators of the PI signaling pathway.

Previous studies from our laboratory using both pregnant and nonpregnant rat myometrial tissue have confirmed that oxytocin and aluminum fluoride activate the PI-signaling pathway thereby stimulating phasic contractions; consistent with the present study, another membrane permeant inhibitor of PI-PLC (ie. 2-nitro-4-carboxyphenyl-N,N-diphenylcarbamate) also significantly suppressed hormone-stimulated phasic contractions in this tissue [11,19]. Consistent with classic calcium oscillation phenomena, the hormone-stimulated phasic myometrial contractions were also found to be significantly inhibited by phorbol ester activation of protein kinase C, nifedipine inhibition of extracellular calcium influx, and removal of extracellular calcium using calcium-free buffer containing 2 mM EGTA [11,18]. The present study demonstrating neomycin inhibition of hormone-stimulated myometrial contractions provides further support for the hypothesis that cytosolic calcium oscillation-like

mechanisms, including activation of the PI signaling pathway, underlie hormone-stimulated myometrial contractions.

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