Inhibition of Oxytocin Induced Contractions in the Rat Uterus by Sialic Acid (*in Vitro*)[1] (36837)

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The sensitivity of the uterus is known to be regulated by estrogen and progesterone, but the nature of the involvement has not been clearly defined. It has been demonstrated in the rat that an inverse relationship exists between progesterone and uterine sialic acid (1) and that a direct relationship exists between estrogen and uterine sialic acid (1, 2). Preliminary work by author (unpublished data) and by Warren and Spicer (3) conflicts with the previous findings (1, 2). The membrane potentials of rat uterine cells were slightly increased with estrogen and greatly increased with progesterone (4). It has been postulated that rat uterine receptors for serotonin contains glycoproteins (5). The importance of glycoproteins in membrane structures has been neglected (6). Since sialic acid is an essential part of membrane glycoproteins and uterine sialic acid can be altered by the ovarian hormones, this experiment was conducted to determine the role glycoproteins might play in controlling the sensitivity of the uterus.

Materials and Methods. Charles River female rats weighing between 150–200 g were given 1 μg/kg of estradiol cypionate[2] 24 hr prior to the experiment. The rats were stunned by a blow on the head and one complete uterine horn was immediately removed. The uterine segment was placed in an isolated tissue bath[3] at 30°. DeJalons (7) solution[4] bubbled with 95% O_2 and 5% CO_2 was used to bathe the tissue.

Oxytocin[5] was diluted from a stock concentration of 20 U/ml to a concentration of 0.2 U/ml. At the required time, increments of either 0.1 ml (0.02 U), 0.2 ml (0.04 U) or 0.3 ml (0.06 U) were diluted to 0.5 ml with DeJalons solution and injected into the tissue bath containing the uterine segment. Since the tissue bath volume was 10 ml, the final concentration of oxytocin was either 2, 4 or 6 mU/ml.

Sialic acid[6] was prepared in concentrations of either 2.5 or 5.0 mg/ml in DeJalons solution. Desired volumes of sialic acid stock solution were selected and diluted to 1 ml. Therefore, a constant volume was always injected into the tissue bath. Final concentrations of sialic acid in the tissue bath ranged from 0.5 to 0.063 mg/ml. Initial tissue bath fluid volume varied slightly but the total final volume was always the same (10 ml) after oxytocin and sialic acid were added to the bath.

The contractions were recorded by a Dynograph[7] with a Force-Displacement Transducer.[8] The variables of maximum amplitude, time from oxytocin injection to maximum amplitude, and time from oxytocin injection to the initiation of the contraction were determined. The velocity of the contraction was calculated during the period of maximum rate of a contraction.

Experiment I. The uterine segment was first stimulated by an injection of oxytocin

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[2] Upjohn Company, Kalamazoo, MI.

[3] Metro Scientific, Farmingdale, NY.

[4] 150.0 m*M* NaCl, 27.7 m*M* glucose, 5.9 m*M* $NaHCO_3$, 0.3 m*M* $CaCl_2$ and 5.83 m*M* KCl.

[5] Jensen-Salsbery Laboratories, Kansas City, MO.

[6] Calbiochem, Los Angeles, CA.

[7] Beckman Instrument, Lincolnwood, IL.

[8] Grass Instrument, Quincy, MA, Model FTO3C.

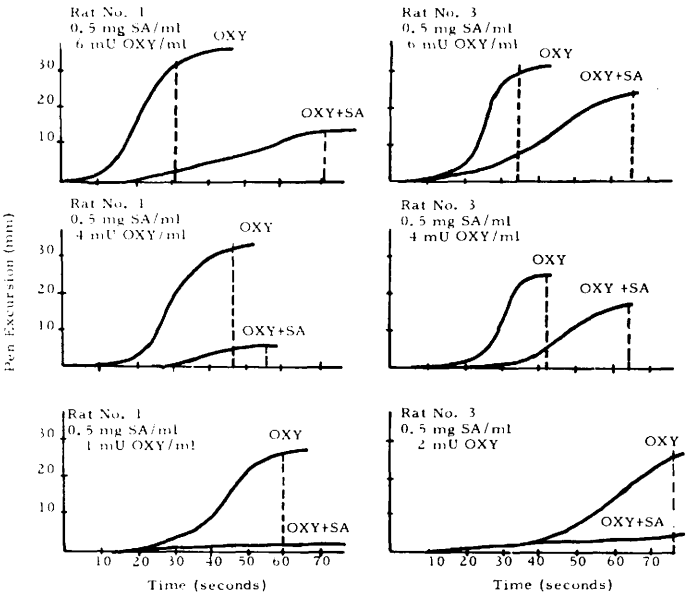

FIG. 1. Exp. I. Comparison of oxytocin and oxytocin-sialic acid stimulated uterine contractions: (- -) bathing solution replacement time, (OXY) oxytocin, (SA) sialic acid.

(OXY-1). When the contraction reached maximum amplitude (Fig. 1), the bathing solution was replaced by continuously flowing, prewarmed oxytocin-free DeJalons solution through the tissue bath for 15 sec. After the uterus relaxed to the preoxytocin base line and a total of 2 min had elapsed from the time of the oxytocin injection, a desired concentration of sialic acid was injected into the bathing solution. Exactly 1 min after the injection of sialic acid, oxytocin was again injected into the bathing solution to stimulate the uterine segment (OXY + SA-2). The tissue bathing solution containing the sialic acid was replaced with sialic acid-free DeJalons solution at maximum amplitude (Fig. 1). Thus, a 3-min interval was maintained between successive oxytocin injections.

Experiment II. The experimental protocol established in Expt. I was altered in two ways. (a) Sensitivity of the uterine segment to the same concentration of oxytocin was tested immediately after each oxytocin sialic acid combination (OXY-3) (Fig. 2), and (b) The time for replacing the bathing solution was held constant for each sequence (OXY-1, OXY + SA-2, OXY-3) instead of allowing each contraction to go to maximum amplitude before flushing.

Results. The data as presented in Fig. 1 demonstrate that 0.5 mg/ml of sialic acid diminishes and delays the contraction responses from 4 and 6 mU/ml of oxytocin. The responses from 1 and 2 mU/ml of oxytocin were negated with 0.5 mg/ml of sialic acid.

Evidence presented in Fig. 2 suggests that the uterus responds normally to the same dosage of oxytocin when it is injected immediately after a sialic acid sequence. The uterine contractions (OXY-1, OXY-3) were (Fig. 2) identical in all of the experiments conducted.

Contrasting the data in Fig. 1 with that in Fig. 2, the OXY + SA-2 contractions reach the same amplitude as the oxytocin contractions. However, the response time and time to peak amplitude are delayed in all OXY+ SA-2 groups. By comparing the uterine responses to 4 and 6 mU/ml at each level (0.25 or 0.50 mg/ml) of sialic acid (Fig. 2), the response time is increased by decreasing the oxytocin. However, if the sialic acid concentration is reduced from 0.5 to 0.25 mg/ml at either the 6 mU/ml (Fig. 2a, c) or the 4 mU/ml (Fig. 2b, d) oxytocin level, there

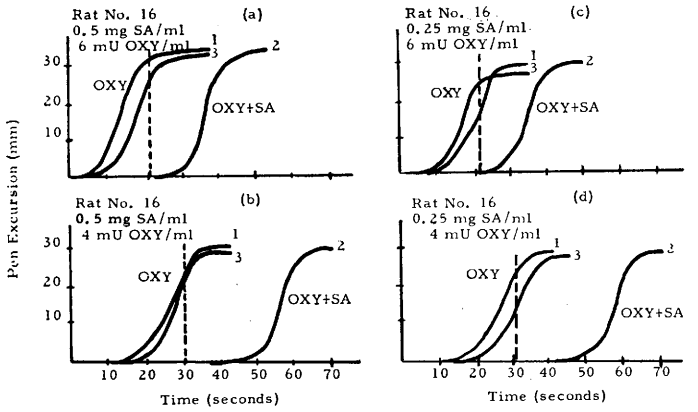

FIG. 2. Exp. II. Comparison of uterine contractions before and after sialic acid treatment: (- -) bathing solution replacement time, (OXY) oxytocin, (SA) sialic acid, (1) presialic acid, (2) with sialic acid, and (3) postsialic acid.

appears to be no difference in the contraction response times.

Although the oxytocin inhibitory properties of sialic acid decreased as the sialic acid concentration decreased, contraction inhibition was evident in solution containing 0.063 mg/ml of sialic acid.

Summary and statistical analyses of data from Expts. I and II (Table I) verify the inhibitory properties of sialic acid on oxytocin for the time to peak amplitude and response time. Since the responses to the 2 mU/ml of oxytocin were negated by the 0.25 and 0.5 mg/ml of sialic acid, the variables could not be calculated for the summary table.

Discussion. The most logical explanation for these inhibitory properties of sialic acid is the formation of an oxytocin–sialic acid complex. The total oxytocin in the 10 ml bath ranged from 20 to 60 mU. The total sialic acid in the bath ranged from 2 to 16 μmoles. Since inhibition appeared to decrease as the oxytocin level increased, the ratio of milli-

TABLE I. Summary of Rat Uterine Contractions Stimulated by Either Oxytocin or Oxytocin and Sialic Acid.

Oxytocin (mU/ml)	Sialic acid (mg/ml)	Time	Maximum amplitude (mm)	Time to maximum amplitude (sec)	Velocity (mm/sec)	Response time (sec)
6	—	Pre	30.3 ± 2.7[a](7)[b]	36.7 ± 2.4 (7)[c]	1.77 ± 0.27 (7)	12.8 ± 2.0 (7)[c]
6	0.5	—	23.4 ± 5.5 (7)	63.7 ± 2.7 (7)	1.34 ± 0.44 (7)	34.0 ± 4.1 (7)
6	—	Post	30.0 ± 4.8 (4)	35.5 ± 4.2 (4)[c]	2.42 ± 0.53 (4)	15.5 ± 5.6 (4)[c]
6	—	Pre	32.8 ± 4.0 (5)	41.0 ± 6.2 (5)[c]	2.38 ± 0.42 (5)	12.3 ± 3.0 (5)[d]
6	0.25	—	29.8 ± 3.5 (5)	61.0 ± 5.3 (5)	2.18 ± 0.30 (5)	33.6 ± 5.7 (5)
6	—	Post	32.2 ± 3.6 (5)	41.0 ± 6.2 (5)[c]	2.44 ± 0.27 (5)	18.4 ± 3.2 (5)[c]
4	—	Pre	27.0 ± 2.4 (7)	46.4 ± 3.3 (7)[d]	1.50 ± 0.19 (7)	18.9 ± 3.2 (7)[c]
4	0.5	—	21.3 ± 3.3 (7)	68.3 ± 6.1 (7)	1.03 ± 0.31 (7)	39.6 ± 6.4 (7)
4	—	Post	25.0 ± 3.0 (5)	43.0 ± 3.0 (5)[d]	1.60 ± 0.27 (5)	21.0 ± 2.3 (5)[c]
4	—	Pre	36.3 ± 2.9 (6)	44.2 ± 4.0 (6)[c]	2.03 ± 0.18 (6)	23.3 ± 3.6 (6)[d]
4	0.25	—	30.3 ± 2.7 (7)	73.9 ± 9.7 (7)	1.93 ± 0.30 (7)	43.1 ± 3.6 (7)
4	—	Post	31.0 ± 3.3 (6)	47.5 ± 2.8 (6)[c]	1.95 ± 0.20 (6)	26.0 ± 3.9 (6)[d]

[a] Mean ± SE.

[b] No. of observations.

[c] Differs from SA treated group: $p < .001$; [d] $p < .01$; [e] $p < .05$.

units of oxytocin to micromoles of sialic acid may provide an indicator for the inactivation of uterine contraction. It appears from these data that if the ratio of oxytocin to sialic acid is around 5 (mU oxytocin/μmoles of sialic acid) inhibition occurs. If the ratio is greater than 5, the inhibitory properties of sialic acid diminishes. Although this ratio is only approximate from the data in these experiments, future experiments may find a more definite ratio.

Another interesting possibility is presented in Fig. 2. If the sialic acid curves were shifted so that the bath flushing time was exactly over the zero time, the oxytocin–sialic acid curves would be identical to the oxytocin curves. This brings up the question, does sialic acid control in some way the membrane deplorization? If oxytocin has set in motion the stimulation of the muscle and sialic acid inhibits the follow through, then washing away the sialic acid allows the stimulation to proceed. This accounts for the similarities in the uterine contraction amplitudes in Expt. II. In Expt. I the bathing solution was not replaced until each contraction had reached maximum height causing an apparent depression of amplitude.

The inhibitory properties of sialic acid on the uterine contractions confirm the involvement of glycoprotein as previously postulated (5). It is also possible that the ovarian hormones influence the uterine sensitivity by regulating the glycoprotein concentration. These experiments demonstrate clearly that the presence of sialic acid in the tissue bath modified the sensitivity of rat uterine tissue to oxytocin.

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