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Oxytocin Receptors: Triggers for Parturition and Lactation?

Abstract. *Specific binding of tritiated oxytocin to uterine receptors of pregnant rats increases dramatically at term and is maximal during labor. In mammary glands the increase in binding is gradual, reaching a maximum during the lactation period. Concomitant changes in the sensitivity of the uterus and mammary gland to oxytocin indicate that the receptor concentration is of functional significance. Oxytocin receptors, therefore, may regulate the response of the target organs to circulating oxytocin and thereby control the onset of labor and lactation. Ovarian steroids participate in the regulation of oxytocin receptors in a manner as yet unclarified.*

The neurohypophysial hormone, oxytocin, causes contraction of uterine smooth muscle and has been used widely in obstetrics to induce labor. Because oxytocin-induced labor cannot be distinguished from spontaneous labor by the pattern of contractions, it was assumed for decades that labor is initiated by the release of oxytocin. Although oxytocin has been found in the maternal circulation throughout pregnancy (*1*), most observations suggest that oxytocin concentrations in the blood increase only during the final stages of labor, and not before (*2*). Therefore, parturition may not be triggered by an increase in oxytocin secretion, but by an increase in the sensitivity of the myometrium of the uterus to the hormone. Several studies, in fact, have shown that the reactivity of the myometrium to oxytocin is maximal at or near labor (*3*, *4*).

We have examined whether the increased sensitivity of the myometrium in pregnant rats is the result of an increase in the concentration of oxytocin receptors in the myometrium at the time of parturition. A sudden increase in the concentration of oxytocin receptors would support the concept that the receptors are the trigger for parturition.

Oxytocin also causes milk ejection in lactating animals by eliciting contractions of the myoepithelial cells surrounding the alveoli in mammary glands. In rats, oxytocin is essential for the removal of milk from the mammary glands (*5*), and the initiation of lactation also may depend on an increase in oxytocin receptors. Therefore, we have determined the concentration of oxytocin receptors in the mammary glands during pregnancy and lactation in the rat.

The specific binding of [^{3}H]oxytocin to particulate fractions from the rat mammary gland and myometrium was estimated as described (*6*). Scatchard analyses indicated that oxytocin was bound to myometrial particles with an apparent dissociation constant (K_d) of 1 to 2 n*M* throughout pregnancy. These values agree with those found in uterine particulate fractions from estrogen-treated rats (*6*). The metabolic breakdown of [^{3}H]oxytocin by uterine particles was generally uniform throughout pregnancy, never exceeding 30 percent after the 1-hour incubation period. The amount of oxytocin bound to myometrial particles was relatively high on day 1 of pregnancy (estrus), but decreased thereafter to near baseline levels by day 5 of pregnancy and remained low until the day of parturition, day 22 (Fig. 1A). Binding then increased rapidly and reached peak values during labor. Binding was re-

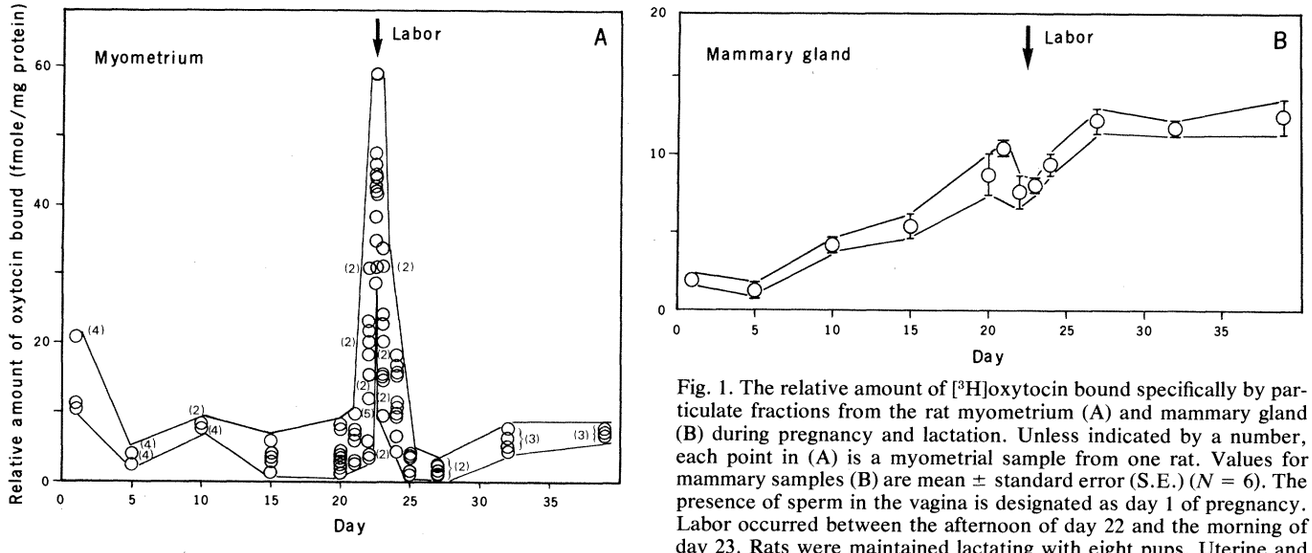

Fig. 1. The relative amount of [^{3}H]oxytocin bound specifically by particulate fractions from the rat myometrium (A) and mammary gland (B) during pregnancy and lactation. Unless indicated by a number, each point in (A) is a myometrial sample from one rat. Values for mammary samples (B) are mean ± standard error (S.E.) (N = 6). The presence of sperm in the vagina is designated as day 1 of pregnancy. Labor occurred between the afternoon of day 22 and the morning of day 23. Rats were maintained lactating with eight pups. Uterine and mammary samples from Sprague-Dawley rats (ARS, Madison) were taken between 1000 and 1200 hours. The myometrium was dissected free and homogenized in nine volumes of Tyrode solution as described (*6*). Particles sedimenting between 1,000*g* (10 minutes) and 48,000*g* (30 minutes) were assayed for binding activity. Each assay tube contained 1 mg of particulate protein, 0.5 n*M* [^{3}H]oxytocin (31 Ci/mmole, 452 I.U./mg, custom synthesized by Schwarz/Mann) in 250 μl of 50 m*M* tris-maleate buffer, *p*H 7.6, containing 5 m*M* $MnCl_2$ and 0.1 percent (weight to volume) gelatin. The amount of [^{3}H]oxytocin bound in the presence of 0.2 μ*M* nonradioactive oxytocin was considered to be nonspecific. Nonspecific binding never exceeded 25 percent of the amount of oxytocin bound specifically during labor. Specific binding was determined by subtracting the amount bound nonspecifically from the total radioactivity. Protein concentrations were determined by the method of Lowry *et al.* (*20*) with bovine serum albumin as the standard.

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duced by the first postpartum day, day 23, and decreased thereafter to baseline levels. The large variation in the amount of oxytocin bound on days 22 and 23 may be due to differences in the time of parturition among individual rats. These results correlate well with the marked increase in response to oxytocin in pregnant rats just before parturition (*4*). The inability of oxytocin to induce delivery in rats earlier than 6 to 8 hours before term (*4*) supports the conclusion that oxytocin receptors are essential for the physiological response.

The binding of [^{3}H]oxytocin to particulate fractions of rat mammary gland increased steadily throughout pregnancy, except for a transient decrease near term, and was maximal during lactation (Fig. 1B). As in the myometrium, the concentration of receptor sites for oxytocin in the mammary gland correlates with the sensitivity of the myoepithelial cell to oxytocin. Sala and Freire (*7*), using mouse mammary strips, demonstrated more than a 40-fold decrease in the threshold dose of oxytocin from day 9 to day 18 of pregnancy, and a further 2.5-fold decrease on days 1 to 10 of lactation.

The importance of estrogens for myometrial sensitivity to oxytocin is well established. Recent evidence indicates that estrogens also regulate the concentration of oxytocin receptors in the myometrium (*8-10*). In ovariectomized rats diethylstilbestrol increases both the concentration of binding sites for oxytocin and the affinity for the hormone within 24 hours of a single injection (*8*). In intact sheep, the concentration of high-affinity sites for oxytocin in the myometrium is highest during estrus (*9*). These observations suggest that estrogens increase myometrial sensitivity to oxytocin by increasing the concentration of oxytocin receptors in the uterus. The finding that plasma estradiol-17β is maximal on the day of parturition (Fig. 2) (*11*) is in agreement with this concept. Csapo (*12*), on the other hand, has suggested that progesterone withdrawal is the main factor in lowering the myometrial threshold for oxytocin. The sharp decline in plasma progesterone prior to delivery supports this view. It is possible that progesterone, an estrogen antagonist (*13*), suppresses the appearance of oxytocin receptors (*10*). Progesterone inhibits the estrogen-stimulated increase in uterine sensitivity to oxytocin in ovariectomized rabbits (*14*) and rats (*15*); but there is little evidence for the inhibitory effect of progesterone on oxytocin sensitivity in other species. The changes in the ratio of plasma estradiol to progesterone most closely paralleled the changes in oxytocin binding (Fig. 2). Our findings indicate that the concentration of estrogen receptors in the myometrium also might be related to oxytocin receptor concentrations. Uterine prostaglandins may modulate oxytocin receptors as well, because uterine prostaglandin $F_{2\alpha}$ enhances the response of the uterus to oxytocin in some species (*16*); although in pregnant rats (*17*) and rabbits (*18*) the evidence is contradictory.

We did not find any obvious correlation between oxytocin binding to mammary particles and plasma estradiol or progesterone concentrations. Because the profile of oxytocin binding in the mammary gland during pregnancy and lactation was very different from that in the myometrium, the two target sites must be regulated differently.

Thus, in rats, parturition is preceded by a dramatic increase in the concentration of myometrial receptors for oxytocin. The high affinity for oxytocin may render the myometrium sensitive to normal levels of circulating oxytocin, which in pregnant women range from below 2 to 24 pg/ml (*2*). The paucity of oxytocin receptors before term provides an explanation for the inability of oxytocin to induce labor in the pregnant rat more than 6 to 8 hours before term (*4*). Although the decrease in the neurohypophysial content of oxytocin during delivery suggests an increase in oxytocin secretion (*19*), parturition may be triggered by an increase in the concentration of myometrial oxytocin receptors apart from an increase in oxytocin levels in the blood. Milk ejection, and consequently lactation, also may depend on a critical level of oxytocin receptors on myoepithelial cells.

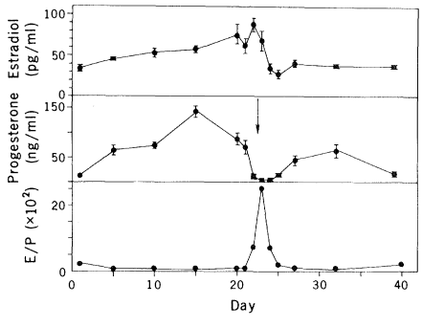

Fig. 2. Concentrations of estradiol-17β, progesterone, and the estradiol progesterone ratio (*E/P*) in the peripheral plasma of rats during pregnancy. Each point is the mean ± S.E. from at least eight rats. Blood was drawn from the abdominal aorta between 1000 and 1200 hours. The concentration of both steroids was determined by radioimmunoassay. For estradiol-17β determinations, plasma samples (2 ml, in duplicate) were extracted with 8 ml of diethylether. Estradiol-17β concentrations were quantitated in duplicate by immunoassay (*21*) with a rabbit antibody to estradiol-17β-6-carboxymethyloxime-thyroglobulin (*22*). The affinities of the antiserum for estrone and estriol were 0.2 and 0.1 percent that of estradiol-17β, respectively. Plasma from ovariectomized, adrenalectomized rats gave blank values of < 19 pg/ml. The values reported were not corrected for the recovery of [2,4,6,7-^{3}H]estradiol-17β (91.3 Ci/mmole, New England Nuclear), which averaged 93 percent in nine experiments. For the determination of progesterone, plasma samples (0.1 ml, in duplicate) were extracted with 4 ml of hexanes (Nanograde, Mallinckrodt) twice. The hexanes extract progesterone, 17α- and 20α-hydroxyprogesterone but not corticosterone and deoxycorticosterone (*23*). The recovery of [1,2,6,7-^{3}H]progesterone (103.7 Ci/mmole, New England Nuclear) was > 98 percent. Progesterone concentrations were quantitated with rabbit antiserum progesterone-11β-succinyl bovine serum albumin (New England Nuclear). The cross-reactivities of 20α-hydroxyprogesterone and 17α-hydroxyprogesterone were 0.4 and < 0.3 percent that of progesterone, respectively. Plasma from ovariectomized adrenalectomized rats gave blank values that were indistinguishable from zero. The interassay coefficients of variation in six experiments with pooled rat plasma were 7.2 and 9.9 percent for estradiol-17β and progesterone, respectively.

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Role of Nuclear Size in Cell Growth Initiation

Abstract. *Swiss 3T3 cells arrested in G_0 (quiescent state) by reducing serum content of the medium all contain the same amount of DNA but vary in nuclear volume over approximately a twofold range. By use of flow microfluorimetry, scatterplots of nuclear volume versus DNA content were obtained in intervals after serum stimulation. The earliest cells to enter DNA synthesis were those with the largest nuclei, whereas cells with the smallest nuclei were among the latest. Regulation of cellular transit from G_0 to the S phase was therefore, at least in part, deterministic, since all G_0 cells did not have equal probabilities of entry into S at a given moment. All cells having the same nuclear volume did not initiate DNA synthesis at the same moment; therefore, factors other than nuclear volume must also influence this timing. Nuclear volume correlated with the maximum rate at which cells could enter S. The kinetic model of the cell cycle postulating a probabilistic event as solely responsible for entry into S thus appears too simple.*

Why individual cells within a culture required widely different time intervals to traverse their own cycle is not known. This variability has been observed with bacteria (*1*), algae (*2*), yeasts (*3*), and animal cells in culture (*4*-*7*). For example, in cultures of Chinese hamster ovary cells, the majority of cells had cycle durations varying from 14 to 21 hours (*4*), which is probably not due to genetic differences since it occurs in freshly cloned populations (*5*). Most variability occurs in the G_1 phase of the cell cycle (*6*-*8*), as does the major regulatory event (*7*, *9*); G_1 variability and cell-cycle control are probably related.

Searches for inherent differences between cells which are responsible for different times of initiating DNA synthesis have focused mainly on cell size. For lymphocytes (*8*) and fibroblasts (*10*), smaller cells require longer to transit G_1 than do larger cells. Similarly, both fission yeast (*3*) and budding yeast (*11*) cells that are below a critical size must grow before initiating their cycle, hence taking longer to complete a cycle than larger cells. Above a minimal size, their cycle time is size-independent. Bacteria also show a dependence of DNA initiation upon cell mass (*12*). These results indicate that the duration of cell-cycle traverse is not purely probabilistic (*7*) but rather is dependent on properties of individual cells.

In this report we examine the relation between *nuclear* size and transit time from the quiescent state to the S phase in contrast to earlier investigators who emphasized *cell* size. Also, we used a different technique. Flow microfluorimetry permits simultaneous determinations of light scattering by nuclei (dependent on nuclear diameter) and DNA content. The results are displayed on a two-dimensional scatterplot of these parameters, in which each point represents an observed cell (see Fig. 1). We can thus observe directly whether cells initially aligned in G_0 enter DNA synthesis at the same time regardless of their nuclear size. In this case, the scatterplot would move parallel to itself to the right. Alternatively, dependence of the timing of DNA initiation on nuclear size would be directly observed, as an earlier movement of larger nuclei, for instance.

Swiss 3T3 cells were grown at 37°C in the Dulbecco modification of Eagle's medium (DME). To align the cells in G_0, an exponential population growing in DME plus 10 percent calf serum was shifted to DME plus 0.5 percent calf serum and left for 36 or 48 hours. The resulting (G_0) population consisted primarily (approximately 95 percent) of cells having a DNA content characteristic of the G_1 phase (Fig. 1a). Determinations of nuclear diameter with a Coulter counter (Fig. 2) showed that all volumes vary over a twofold range. The initial (G_0) population's scatterplot obtained by flow microfluorimetry (Fig. 1b) showed this nuclear volume heterogeneity in contrast to the uniform DNA content of most of the cells. Growth was initiated by replacing the medium with DME plus 10 percent calf serum. At subsequent times nuclei of replicate cultures were analyzed by flow microfluorimetry (*13*, *14*) to obtain both DNA histograms and scatterplots of nuclear size (diameter) versus DNA content. The nuclear sizes are on an arbitrary scale.

After addition of complete medium to G_0 cells, significant numbers of cells had entered the S phase by 18 hours, some of which reached the G_2 phase (Fig. 1c). These 3T3 cells have a minimal G_0-to-S interval of approximately 11 hours and an S period of 8.5 hours (*14*). The scatterplot (Fig. 1d) of this 18-hour population shows that most cells of the smallest nuclear size had not yet increased their DNA content. Among cells of progressively greater nuclear size, progressively greater fractions had entered farther into S. Primarily, only the cells with the largest nuclei had acquired G_2 DNA by 18 hours.

Scatterplots made at other times reinforced the conclusions from the 18-hour data. The scatterplot made at 14 hours had a similar but narrower wedge shape than shown in Fig. 1d. After serum stimulation for 20 hours (and 22 hours, not shown), more cells with smaller nuclei acquired a G_2 DNA content (Fig. 1, e and f). Since cells with the smallest nuclei eventually synthesized DNA, nuclear size was not a necessary constraint on entry into S. The size of the arrested cells' nuclei thus correlated positively with the maximum rate at which the cells responded to serum stimulation.

An important feature of these data is that cells of a given nuclear size did not all transit to S in the same time. Had they done so, one would have seen a narrow

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