Binding of Oxytocin to Uterine Cells in Vitro

OCCURRENCE OF SEVERAL BINDING SITE POPULATIONS AND REIDENTIFICATION OF OXYTOCIN RECEPTORS

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Myometrial and endometrial cells of sheep, rat, and calf in monolayer cell culture display at least three populations of binding sites for oxytocin, with dissociation constants (Kd) of approximately 5 x 10^{-9}, 4 x 10^{-7}, and >10^{-5} mol/liter, respectively. Binding of the tritium-labeled oxytocin (concentration range, 10^{-11} to 5 x 10^{-4} M) to the first two sites is displaceable by cold oxytocin. The ratio of binding capacities of the high to medium affinity site appears to average 1:18. Dissociation rate constants for these sites (22 °C) are roughly 10^{-4} and 2 x 10^{-5} s^{-1}, respectively. The capacity of the low affinity site varies in individual cell preparations and is between 5 and 66 times that of the medium affinity site. The low affinity binding sites may not be fully saturable and may follow a non-symptotic binding isotherm. Logarithms of Kd and binding capacity for individual binding sites are linearly correlated. The coexistence of the three sites was also proven by cluster analysis based on similarities between Kd, binding capacity, and Hill coefficient. Only minor systematic species and cell type differences occur in these properties. The value of Kd for the oxytocin receptor in rat myometrium, derived recently from a stepwise irreversible inhibition of uterotonic response to oxytocin, is close to 2.5 x 10^{-7} mol/liter. Additional pharmacological data (pA2 values of structural analogues of oxytocin acting as competitive inhibitors) also reveal a Kd value of 3 x 10^{-7}. It is, therefore, concluded that the receptors for oxytocin in rat myometrium are identical with the medium affinity site.

The interaction of oxytocin with its receptors in the rat, sheep, and human uterus has recently been the subject of several studies. In all of them, the dissociation constant of the intermediary hormone-receptor complex (Kd), in the presence of high Mg²⁺ and Mn²⁺ concentrations (up to 10 mM), has been reported to be about 2 x 10^{-2} mol/liter (1-5). This value has been considered to be very likely, since the concentration of oxytocin which causes a half-maximal response on the isolated rat uterus strip (EC₅₀) is numerically similar. Additional pharmacological evidence, however, indicates a considerably weaker binding. So, for instance, Kd resulting from dose-response analysis after partial irreversible inhibition of the receptors on isolated rat uterus is approximately 2.5 x 10^{-7} mol/liter (6, 7). Similar values have been obtained from analysis of IC₅₀ values for oxytocin antagonists structurally analogous to the parent hormone (8). Also, oxytocin binding to the sites with Kd ≈ 10^{-8} mol/liter lacks any correlation to dose-response profiles when comparing normal and Brattleboro strains of rats (hereditary neurohypophyseal insufficiency), at least for one reported line of these animals (9).

These discrepancies caused us to re-examine the binding isotherm of oxytocin on myometrial and endometrial cells. In order to avoid binding on sites that are inaccessible to the hormone in intact tissue and to better mimic the physiological conditions, we have carried out our experiments on dissociated and shortened cultured cells, at magnesium concentrations similar to extracellular values.

MATERIALS AND METHODS AND RESULTS

Model of the Binding Equilibrium and Computations—Relation between bound and free concentrations of a ligand at a constant concentration of the binding macromolecule (and at a constant temperature) is referred to as “binding isotherm.” For current models of binding equilibria on one single binding site population (i.e. a population in which binding energies of individual sites, and consequently also Kd values, are distributed around a single mean value according to a distribution law of statistical thermodynamics), such a binding isotherm is frequently approximated by the equation

\[ c_b = B_0/(K_d/c_h)^h + 1, \]

where \( B_0 \) is the binding capacity of the site and \( h \) the Hill coefficient. Mutual interaction of the sites within this population is reflected in the value of \( h \) (\( h \neq 1 \) for sites displaying cooperative behavior). Parameters in Equation 1 can be assessed by optimization of the power coefficient \( h \) within a preselected interval. The procedure has been described earlier (15).

An isotherm which describes binding of a ligand in a system with several binding site populations (a common situation in any biological system) is formulated as a sum of terms corresponding to individual populations. Thus, when Equation 1 is applicable to all populations of such a multisite system,

\[ c_b = \sum_{j=1}^{n} B_{0,j}/((K_{d,j}/c_h)^h + 1); \]

1 Portions of this paper (including "Materials and Methods," part of "Results," Figs. 3-6, Tables 1 and II, and Equations 6-13) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 86M-2289, cite the authors, and include a check or money order for $5.20 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

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j-indexed symbols stand for parameters of the individual sites. A suitable numeric method for estimating parameters in Equation 2 is lacking. However, in a narrow $c_j$ range around a certain $K_{d,j}$, Equation 2 can be reduced to

$$c_j = B_0 + B_{m,j}((K_{d,j}/c_j^m + 1),$$

under the conditions that all binding sites with $K_{d,j} < K_{d,k}$ are already saturated and that the binding on sites with $K_{d,j} > K_{d,k}$ is minimal within the $c_j$ range in question. The linearized form

$$c_j/c^m = (B_0 + B_{m,j})/K_{d,k} - c_j/K_{d,k} + B_{m,j}/c^m$$

enables optimization of the parameter $h$, and computation of parameters $B_0, B_{m,j}$, and $K_{d,j}$ in a similar way as for Equation 1 (15). The rearranged linearized form of Equation 3, that employs optimized $B_0$ and $h$, values, assigned here as the "linearized Scatchard plot."

$$c_j - B_0/c^m = B_{m,j}/K_{d,k} - (c_j - B_0)/K_{d,k},$$

yields straight lines only within $c_j$ ranges around individual $K_{d,j}$ values (16); in a broader $c_j$ range, the plot is curvilinear (Fig. 1). Vice versa, linear segments in Equation 5 can be employed for computation of the binding parameters. To find these segments, the data were ordered in a series with ascending $c_j$ values. The shortest sequence investigated contained five points and was successively extended by one point each time. Thus, the fit was carried out with points 1 through 5, 1 through 6, etc. up to 1 through $n$, then with points 2 through 6, 2 through 7, etc., until all subseries $(n - 3)$ ($n - 4/2$ in number) were computed. The computation itself consisted of two steps: (i) the estimate of constants $K_{d,j}, B_{m,j}, B_0$, and $h$ as mentioned above; (ii) computation of the correlation coefficient $r$ for linearized Scatchard plot (Equation 5). Optionally, the points within the groups can undergo smoothing by a polynomial of second to fourth degree. Distinct populations can be identified when the ratio of their $K_{d,j}$ values (larger-to-smaller) is at least 50-100. An example is shown in Fig. 2.

The results indicate that $K_{d,j}$ values obtained from those sequences of points which give an approximately linear plot according to Equation 5 appear only within certain "peaks" (right-hand panel). These peaks obviously represent individual populations of binding sites; their mean binding parameters (geometric means) were computed from all groups of data points which yield a significantly linear plot according to Equation 5 (at least on the 5% probability level). The plot of related $K_{d,j}$ and $B_{m,j}$ values, called the "affinity spectrum" (17) of the system (Fig. 2, lower right), shows the binding capacity of these sites.

Analogous binding profiles were obtained by the recently published "affinity spectrum method" which detects, in certain instances, individual binding site populations even more directly (17). These computations were performed by Dr. H. J. Tobler, Sandoz AG, Basel, Switzerland.) The method also enables an estimate of the so-called "nonspecific binding" (operationally, the binding which is not displaceable by an excess of "cold" ligand), in this case less than 0.04% of the total. It neglects, however, possible inequality of their $h$ values. The upper left-hand panel in Fig. 2 demonstrates that the fit yields differing $h$ values for these sites.

**DISCUSSION**

Sheep, rat, and calf myometrial cells carry several types of binding sites for oxytocin. Those sites differ in their thermodynamic and kinetic properties and most probably in biochemical properties (e.g. metal activation (3)) as well. The binding capacities of the three populations identified by analysis of the binding isotherm were found to correlate, without any known cause, with the equilibrium dissociation constants (Fig. 4). Displacement of oxytocin from the two sites with highest affinity follows the predicted model (Equation 6), whereas the linearization by Equation 10 failed for the third site, for which $K_d = 10^{-8}$ mol/liter (cf. Table I). The low affinity site displays apparent departures from the regular displacement process; the population may consist of "nonspecific," although perhaps saturable, binding elements, which bind oxytocin in a noncomplementary way.

The phenomenon of distinct classes of binding sites on target cells is not uncommon for neurohypophysial and also other peptide hormones. Two distinct classes of binding sites for oxytocin have already been found on rat epididymal adipocytes (21); they seem to initialize antagonistic effects on glucose uptake and metabolism in these cells (22). Their $K_d$ values, $1.1 \times 10^{-9}$ and $7.5 \times 10^{-10}$ mol/liter, are very similar to those on myometrial cells (vide supra). Arginine vasopressin was also reported to bind to two binding sites in rat brain membranes (23), with $K_d$ values of $4.2 \times 10^{-10}$ and $1.3 \times 10^{-8}$ mol/liter, the lower one only being detected at pH 8 after repeated freezing and thawing of the membranes, or by adding 5 mM NiCl$_2$. Despite the complex pH and metal activation that may cause difficulties in interpretation of binding profiles, the coexistence of several binding sites can be proven also in this instance.

**Fig. 1.** Linearized Scatchard plot: binding experiment with sheep myometrial cells cultured for 6 days. Left-hand panel, values in a broad concentration range smoothed by a polynomial (4th degree); connecting line represents spline interpolation (note the semilogarithmic plot). Right-hand panel, $c_j$ region 0.62 to 26.5 nM (linear least squares fit). Computed parameters: $K_d = 5.0 \times 10^{-8}$ mol/liter, $B_0 = 7.1$ pmol/mg of protein, $h = 1.9$. 
Experiments carried out in a narrow concentration interval depart from the assumed models. Receptor internalization is essentially identical in all instances; the answer to the first part of the question posed above is, therefore, definitely no, to the second part basically no.

Several circumstances may indeed account for a heterogeneity which might be of minor importance, e.g. associated with larger departures from the assumed models. Receptor internalization, for instance, may represent such a deviation. Although not yet identified in the uterine cells, it is rather likely to exist there, and if so, it may exercise an effect on steady-state measurements of $K_d$ which is not fully predictable. On the other hand, several observations, including the similarity of intact myometrial cells and their membrane preparations with regard to the binding site profiles, speak circumstantially against a major influence of this potential mechanism.

Which binding sites may now be delineated as receptors in the pharmacological sense, i.e. units which initiate a cellular response? It became almost common practice to rest the proof of identity of the $K_d$ and the dose eliciting the half-maximal response ($EC_{50}$). This argument is apparently incorrect since there is no simple relation between the $EC_{50}$ and $K_d$ values (25, 26), let alone direct numeric correspondence. Kinetic proof for this statement can be provided by the analysis of stimulus-response coupling for drug-induced responses (26). In qualitative terms, however, a simple and long known experiment demonstrates it quite lucidly: when the total number of receptors in a responding biological system is stepwise decreased by irreversible inhibition, the $EC_{50}$ value increases while the $K_d$ value should remain unchanged (27). This phenomenon of "receptor reserve" is also known for the response of uterine to oxytocin (28, 29), its $EC_{50}$ value corresponding to a subtotal inhibition of oxytocin receptors is around $10^{-7}$ M, compared to $10^{-9}$ M in noninhibited muscle. Having used an improved method (7) by Furchgott and Burszyn (6), we have computed an average $K_d = 2.5 \times 10^{-7}$ mol/liter from these partially inhibited responses. This allows the conclusion that the in vitro uterotonic response to oxytocin in rat, at low magnesium concentrations, is triggered on these sites. There are not great differences in binding for the three species investigated, and it is likely that a similar conclusion also holds for calf and sheep uterus which have not yet been thoroughly investigated pharmacologically. Rather low affinity for oxytocin is indeed not astonishing when one considers the effect of receptor reserve resulting from amplification within the stimulus-response coupling. In fact, under these circumstances, high affinity binding would appear uneconomic from both thermodynamic and regulatory viewpoints. The system discussed here is certainly no exception in this respect, and low affinity hormone-receptor binding may be a very common feature.

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REFERENCES

1. Soloff, M. S. (1975) Biochem. Biophys. Res. Commun. 65, 205–212
2. Soloff, M. S. (1976) in Hormone-Receptor Interactions—Molecular Aspects (Levey, G. S., ed) pp. 129–151, Marcel Dekker, Inc., New York
3. Soloff, M. S., Schroeder, B. T., Chakraborty, J., and Pearlmutt, F. (1977) Fed. Proc. 36, 1861–1866
4. Crankshaw, D. J., Brands, L. A., Matlib, M. A., and Daniel, E. E. (1978) Eur. J. Biochem. 86, 481–486
5. Fuchs, A.-R., Fuchs, F., Husslin, P., Soloff, M. S., and Fern-
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19697

18. Troyon, R. C. and Bailey, D. E. (1970) Cluster Analysis, McGraw-Hill Publications, Minneapolis, MN

19. Engelmann, L. (1983) in BMDP Statistical Software—1983, printing with Additions (Dixon, W. J., ed) pp. 456–463, University of California Press, Berkeley, CA

20. Foss, S. D. (1970) Biometrika 57, 515–521

21. Sonnert, D. and Cohen, B. (1975) Eur. J. Biochem. 54, 295–303

22. Muchmore, D. B., Little, S. A. and de Haen, C. (1981) J. Biol. Chem. 256, 365–372

23. Junig, J. T., Abooob, L. G. and Skrobla, A. M. (1985) Neurochem. Rev. 10, 1187–1202

24. Mannoni, P. J. and Redbard, D. (1980) Adv. Biochem. 107, 220–239

25. Furchgott, R. F. (1966) Adv. Drug Res. 3, 21–55

26. Lindeberg, G., Vilhardt, H., Larsson, L. E., Melin, P. and Pliika, V. (1980) J. Recept. Res. 1, 399–402

27. Nickerson, M. (1956) Nature 178, 697–698

28. Pliika, V., Marbach, P., Väsiä, J. and Rudinger, J. (1977) Experientia 33, 367–369

29. Pliika, V. and Marbach, P. (1978) Eur. J. Pharmacol. 49, 213–222

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**SUBLINGUAL MATERIAL**

**TO BINDING OF OXYTOCIN TO UTERINE CELLS IN VITRO OCCURRENCE OF SEVERAL BINDING SITE POPULATIONS AND RE-IDENTIFICATION OF OXYTOCIN RECEPTORS**

by

F. Pliika, J. Nehting, N. Miller-Heimths, P. Pliina and E. Elberg

**MATERIALS AND METHODS**

Culture of starting cells

Rat, sheep and calf myometrial and endometrial cells were kept as short-term monolayer cultures (7 to 21 days) in Ham's F-10 medium containing calf serum and L-glutamine (Seromed, München, FRG). Cells were obtained from young females (6-8 weeks for rats [10], 6-8 months for lambs [11] and calves). Colcemid was scraped off and myometrium was separated from the perimetrium membrane. The washed tissues were incubated in phosphate buffered saline pH 7.4 (PBS) containing 0.002% collagenase and 0.02% trypsin (1 g per g tissue) for 30 min at 37°C. The supernatant was decanted and the incision was repeated for 30 min with fresh enzyme solution. Fused supernatants were filtered through a cellulose cone, the cells were collected by centrifugation (600 g) and washed twice after resuspension in 1.5 ml medium containing penicillin, streptomycin and fungicide (Bacto-Thyox, East Germany, FRG). The cells were cultured in this medium for one hour at 37°C (incubating period). Quickly adhering cells which remained on the bottom of the tissue culture flask showed morphological features of fibroblasts. The supernatant containing slowly adhering cells was transferred into another tissue culture flask and incubated at 37°C in a CO₂ atmosphere. The medium was changed every third day. The supernatant cells adhered in clumps, rounded elements, arranged in parallel bundles on the bottom of the flask. The endometrial cells adhered less tightly, with a regular arrangement of single cell-to-cell contact. Shortly before the binding experiment, cells were liberated from the bottom by a 10 min incubation with 0.25% trypsin (BPHP), washed twice with the medium and resuspended in PBS. Alternatively, incubation for 20 min at 0.05% collagenase, or mechanical scraping of the cells from the bottom of the tissue culture flask using a scraper with stainless steel blades, were employed. Dead cells were counted after trypan blue staining: 30–40% of the cells were viable.

Total protein concentration in the cell suspension was measured in small aliquots after centrifugation in an Eppendorf centrifuge (5 min, 10,000 rpm), washing with PBS and dissection of the pellet in 0.1 M NaCl hydroyde, by the method of Lowry et al. [12]. A calibration curve with desiccated bovine serum albumin as reference was constructed under equal conditions (higher concentration of somatic protein than is original protein yield).

Purified Oxytocin

Oxytocin titrated on positions 2 and 5 of 7-hydroxy was prepared in collaboration with American International, Compagnie (France), by catalytic reduction of the amino-terminal peptide [13]. The titrated peptide purified on a reversed-phase HPLC column showed a specific radioactivity of 20% of total. Preparations with specific radioactivities of 6-20 Ci/mmol, obtained by thinning with cold oxytocin, were used in binding studies. The mixture was kindly donated by FERRING Pharmaceuticals, Helmond (Netherlands).

**BINDING EXPERIMENT**

An equilibrium binding experiment was carried out in a series of 12 Eppendorf cuvettes, each containing the equal number of cells (averaging 2 x 10⁶ cells/100 ll) in 0.2 ml of Hepes buffered physiological salt solution (HBSS): NaCl 145 mM,

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21. Sonnert, D. and Cohen, B. (1975) Eur. J. Biochem. 54, 295–303
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In the presence of a competitive inhibitor (10), the modified eq. 1 runs

$$
C_0 = \frac{B_0}{K_d} \left( 1 - e^{-K_d C} \right) \left( 1 + K_d C \right), \tag{11}
$$

where $C_0$ is the tracer concentration in the absence of the receptor, $K_d$ is the dissociation constant of the receptor-inhibitor complex and $B_0$ is the corresponding Hill coefficient. Assuming that the tracer and the competitive inhibitor (syractide and "cold" oxytocin, respectively) interact with the same receptor, dissociation constants and Hill coefficients are equal ($B_0 = K_d = K_d = 1$), so that eq. 6 becomes

$$
C_0 = \frac{B_0}{K_d} \left( 1 - e^{-K_d C} \right) \left( 1 + K_d C \right), \tag{12}
$$

The relative displacement $F$, i.e., the ratio of the bound concentrations in the presence and in the absence of CI, is

$$
F \equiv F(C) = \frac{B_0}{K_d} \left( 1 - e^{-K_d C} \right) \left( 1 + K_d C \right), \tag{13}
$$

with $B$ and $F$ being bound and free concentrations in the absence of CI ($C = 0$), respectively. The total tracer concentration must be naturally equal: $C_T = C + F = B$. The total concentration of CI can be inserted for $C_T$ when $C = 0$.

The displacement experiments furnish $C_T$ and $F_T$ values for a certain $C_T$ (e.g., differences for variable $C_T$'s are however small when $C_T = C$), and the concentration $F$ and $B_T$ can be evaluated by various computational procedures; two of them have been applied in our experiments:

1) Evaluation of $F$ by the Fitzsimons' strategy similar to that mentioned above (14). A transformed form of eq. 7

$$
(F - 1) = \frac{(C_T - C)}{(C_T - C_0)} \frac{(C - C_0)}{(C - C_0)} \tag{14}
$$

was employed; constants $k_T$ and $k_0$ for a fixed $C_T$ were computed by the least squares method, after corresponding $C_T$ (eq. 7) transformation. The $C_T$, $k_T$ data for $C_T = 0$ can also be employed.

2) In most of the cases $C_T/F = 1$. Eq. 8 then yields

$$
log \left( \frac{F}{F - 1} \right) = \log \left( \frac{k_T}{k_T - 1} \right), \tag{15}
$$

When $F$ is constant for all $C_T$, $k_T$ and $k_0$ can be computed, for instance by least squares method. This procedure was routinely used in our displacement experiments (see Fig. 5).

Computed $k_T$ and $k_0$ values are, for a constant series of competitive concentrations (15, Method), function of tracer concentration. Comparison with $k_T$, $k_0$ obtained by direct binding of tracer indicates a remarkable similarity in both numeric values and concentration dependence (Fig. 5).

The cells of the quickly sediments fraction yield an irregular displacement results with no systematic $C_T$-dependence. The analysis of displacement described by eq. 10 indicates no significant regression in all our cases we investigated.

Wash-out experiments

A mathematical model of the concentration of dissociated tracer to the receptor ($C_0$) in the presence of a high concentration of cold oxytocin (probability of back-binding of the tracer is very low) follows a multiple exponential time course.

$$
C_0 = \frac{B_0}{K_d} \left( 1 - e^{-K_d C} \right) \left( 1 + K_d C \right), \tag{16}
$$

$C_0$ is the initial tracer concentration at time $t$, $B_0$ is the initial bound concentration, $K_d$ is the dissociation rate constant, and $p$ is the number of classes (see eq. 2).

An extended model described by

$$
C_0 = \frac{B_0}{K_d} \left( 1 - e^{-K_d C} \right) \left( 1 + K_d C \right), \tag{17}
$$

was an experiment in which $A$ is the portion of the tracer which was present in the free form at time zero (complete removal of supernatant). Exponential analysis (multiple exponentials) was carried out by the integration of the characteristic equation derived from the final expression (eq. 16 or 17), as suggested earlier (20).

The non-displaceable fraction is defined as

$$
N = 1 - \frac{B_0}{K_d} \left( 1 - e^{-K_d C} \right) \left( 1 + K_d C \right), \tag{18}
$$

where $B_0$ is the total bound concentration at $t = 0$. $C_{T,max}$ is the final $C_T$ obtained in the experiment. If the amount of tracer bound after the final wash-out step (corrected, if possible, for the loss of cells during the wash-out) is the value $C_{T,max}$, then model for applied for eq. 16, $N$ is set to zero. In all our wash-out experiments, the non-displaceable fraction was insignificantly low ($N < 0.5$) over 90% of the tracer was bound reversibly on the cell surface.

Experiments with whole myometrial cells suggest a presence of two binding sites populations on the slowly adhering cells, differing in their $K_d$ values (Tab. II). Both constants are independent of the initial tracer concentration, fraction $A_1/A_2 + A_2$, however, in decreasing with the increasing linear concen-

![FIG. 3. Oxytocin binding to smooth muscle cells: dependence of $A_1$ (mol/L) upon $C_T$ (mg).](image)

![FIG. 4. Oxytocin binding to smooth muscle cells: relationship between $A_1$ (mol/L) and $C_T$ (mg/mg myonephron) for binding site populations found in individual experiments. Circles denote three clusters identified with cluster analysis. Circle markers: maximum variance distance between points within each cluster and the arithmetic mean of the cluster (closed points).](image)

![FIG. 5. Displacement experiments with smooth muscle cells. Concentrations of tracer: 1.1 x 10^{-10} M, closed points (controls): $K_d = 1.43$ x 10^{-10} mol/L, 2.1 x 10^{-10} M, open points (controls): $K_d = 2.36$ x 10^{-10} mol/L). Data transformed according to eq. 10.](image)
### Table I

Oxytocin binding to uterine cells: parameters of binding sites identified in various Kd regions

| Type of cells | Species | N | Kd (nM) | Kd (nM) | Kd (nM) | Kd (nM) |
|---------------|---------|----|---------|---------|---------|---------|
| Cervical cells | Human | 67 | 2.45 ± 0.45 | 1.65 ± 0.25 | 1.05 ± 0.15 | 0.65 ± 0.10 |
| Uterine cells | Human | 67 | 3.85 ± 0.45 | 2.65 ± 0.25 | 2.05 ± 0.15 | 1.55 ± 0.10 |

* Kd is the dissociation constant, nM = nanomolar. Values are mean ± SEM. Numbers in brackets refer to the number of experiments.

### Table II

Binding of oxytocin to sheep uterine cells: rate constants of dissociation (Kd) and association (ka)

| Parameter | High affinity | Medium affinity | Low affinity |
|-----------|---------------|----------------|-------------|
| Kd (nM)   | 4.5 ± 0.2 (3) | 3.1 ± 0.1 (3) | 2.0 ± 0.1 (3) |
| ka (s^-1) | 2.1 ± 0.2 (3) | 1.1 ± 0.1 (3) | 0.6 ± 0.1 (3) |

* Kd is the dissociation constant, nM = nanomolar. Values are mean ± SEM. Numbers in brackets refer to the number of experiments.

* a - 10^-9, b = 10^-9

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**Figure 6.** *Initial experiments.* Change of pre-exponential constants A obtained by a double exponential fit with the initial tracer concentration (C0) used in the binding experiment. Constant A is expressed in percent of the total A0.