Progesterone sperm chemoattraction may be modulated by its corticosteroid-binding globulin carrier protein

Progesterone, the main steroidal component secreted by the cumulus cells that surround the egg, chemotactically guides human spermatozoa. The aim of this work was to evaluate whether the carrier protein corticosteroid-binding globulin also participates in the sperm P chemotactic response. By means of videomicroscopy and image analysis, we observed that corticosteroid-binding globulin modulates the chemotactic activity of P, when a solution of corticosteroid-binding globulin + P is at the nanomolar range. (Fertil Steril® 2010;93:2450–2. ©2010 by American Society for Reproductive Medicine.)

Sperm chemotaxis is a cell transport mechanism that guides spermatozoa toward the source of an attractant concentration gradient (1). In mammals, the chemotactic response was characterized in spermatozoa of several species, such as humans (2, 3), mice (4, 5), rabbits (6), and bulls (7), toward different physiological attractant sources (follicular fluid [FF], oviductal fluid, and conditioned medium from the egg–cumulus complex).

Recently, we observed that P—the main steroid of the egg microenvironment—attracts human and rabbit spermatozoa mainly at the picomolar range (1–100 pM) and to a lower extent at the 1-μM steroid concentration (8). We also found that the chemotactic response mediated by P is correlated with the incubation time for capacitation, suggesting that only capacitated spermatozoa are able to respond to P (8). However, a stable attractant concentration gradient must be formed for chemotaxis to occur in vivo. The cumulus cells synthesize and secrete P (9), where the viscosity of the cumulus extracellular matrix keeps a stable chemoattractant gradient. By means of immunocytochemistry and confocal image analysis we observed that the cumulus cells producing P are radial distributed, being closer to each other near the egg surface. Thus, when the hormone is secreted from the cumulus cells, the cell distribution favors a gradual P gradient formation from the center to the periphery of the cumulus cellular mass (8). In addition, we demonstrated that P is the sperm chemoattractant secreted by the cumulus cells (10). These results support the possibility that the cumulus oophorus and its surrounding may be a potential site of P-mediated sperm chemotaxis during human fertilization.

Although we have characterized the chemotactic response of human spermatozoa toward free P, in biological organisms the steroid hormone is transported by carrier proteins, a corticosteroid-binding globulin, generally referred to as CBG (11). This protein belongs to the superfamily of SERPINs (serine proteinase inhibitors) and functions as a carrier for corticosteroids (12). The CBG, which is also synthesized and secreted by cumulus oophorus cells (13), binds P in a reversible way with an affinity constant (K_d) of about 3×10^{-8} M (14). Similarly to P distribution, by the time of ovulation, the CBG–P complex may be gradually distributed along the cumulus. The aim of this work was to evaluate whether the P carrier protein CBG also participates in the sperm chemotaxis response, and if so, to what extent.

Human semen sample assays were performed in accordance with the guidelines for experimentation of the Declaration of Helsinki. The chemotaxis assays were carried out with spermatozoa previously capacitated and then exposed to a wide concentration range (femtomole to micromole) of P, CBG, or a solution of CBG + P, determining the percentage of oriented spermatozoa, as previously described (6, 10). Briefly, the experiments were performed in a chemotaxis chamber that consists of two wells separated by a 2 mm wall, one filled with medium with or without attractants and the other, with spermatozoa. The chamber was sealed with a coverslip, thus a capillary space (called bridge) was formed between the wells and the separating wall. Across the bridge, a one dimension attractant concentration gradient was formed in the direction of the well containing the spermatozoa, which in turn, swam up over the bridge. Fifteen minutes after sealing the chamber (time necessary to stabilize the sperm distribution and the attractant gradient), the sperm movement was recorded along the fields in the middle of the bridge. Then, the

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sperm tracks were analyzed by videomicroscopy and computer image analysis to calculate chemotaxis.

For each sperm track, the distance traveled along the X axis (representing the attractant gradient; DX) and the Y axis (representing the absence of attractant gradient; DY) were calculated. Assuming that a chemotactic spermatozoon travels a longer distance along the X axis than in the Y axis, sperm directionality was calculated by the quotient DX/|DY|. When this value was >1, the spermatozoon was considered oriented toward the attractant well. As negative control, a culture medium without attractants was loaded where ~25% of spermatozoa swimming at random are expected to be oriented toward the well without attractants. The chemotactic responding subpopulation was considered as the difference in the percentage of "oriented spermatozoa" between the attractant solution and the negative control.

Because the chemotactic response is strongly dependent on the attractant concentration, several doses of the attractant solution were assayed. Thus, a bell-shaped curve, typical of any chemotactic cell is observed, where at low attractant concentration there is not enough receptors stimulated, but they are saturated at a high attractant concentration (15). As a consequence, in both extreme cases the chemotaxis response is abolished and the level of oriented spermatozoa is similar to the basal negative control (~25%). In contrast, at optimum attractant concentration the cells are able to sense the gradient and respond with a chemotactic movement orientation, giving a level of oriented spermatozoa statistically higher than the basal negative control. Because in mammals such a difference is ~10%, a high number of spermatozoa per treatment must be analyzed (minimum 300 cells), in at least three experiments.

When capacitated human spermatozoa were exposed to a concentration gradient of P, we observed a significant chemotactic orientation at the picomolar range (1–100) and at a lower extent at 1 μM P (Fig. 1A), in agreement with previous results (8). Then, we further investigated the chemotactic potential of CBG alone or combined to P. There was a significant chemotactic response when human spermatozoa were exposed to a solution of CBG + P at two concentration ranges 1–100 pM and 10 nM (Fig. 1B). However, when spermatozoa were exposed to a gradient of CBG, no chemotactic activity was observed along the wide range of concentrations assayed (Fig. 1C).

Considering the dynamic kinetic of the steroid binding to its carrier protein, for each concentration of the CBG + P solution, we calculated the concentration of bound and unbound P according to the formulas:

$$[bound\ \ P] \cong \frac{[CBG]_i\ x[P]_i}{K_d}$$

$$[unbound\ \ P] \cong [P]_i - [bound\ \ P]$$

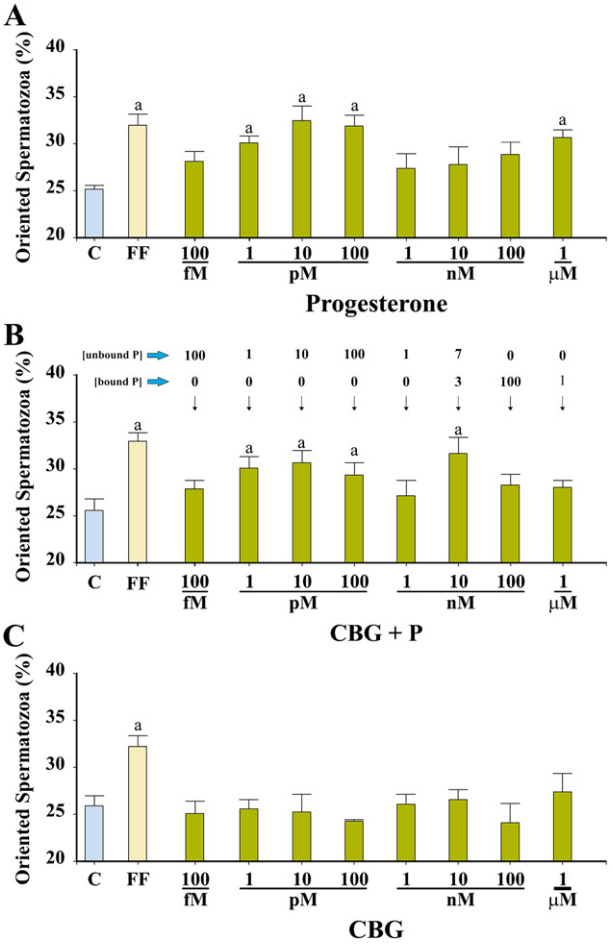
where, $K_d = 3 \times 10^{-8}$ M, $[CBG]_i$ = initial concentrations of CBG, and

$$[P]_i = \text{initial concentration of P.}$$

Because CBG has a binding affinity to P of 3×10^{-8} M, the equilibrium concentration of bound and unbound P is expected in the same concentration range (14). With this scenario, at 10 pM (or lower concentration) of the CBG + P solution, most P is unbound to CBG (see estimated concentration in the upper part

FIGURE 1

Percentage of oriented spermatozoa exposed to a wide range (femtomole to micromole) of P (**A**), corticosteroid-binding globulin (CBG) + P solution (**B**), and CBG (C). The estimated quantities of unbound and bound P per each concentration of CBG + P solution are shown in the upper portion of **B**. Data were calculated according to the formulas included in the text, and values were rounded up for clarity. A 10-mM stock solution was prepared for P (Sigma-Aldrich, St. Louis, MO) and CBG (Fitzgerald, Frankfurt, Germany) in dimethyl sulfoxide (DMSO) and deionic water, respectively. Both compounds were kept frozen at -20°C until the day of the experiment, when they were mixed in equimolar quantities. For each treatment, a total of 300-3,000 spermatozoa were analyzed in 3-10 experiment repetitions. Differences between treatments were determined by a one-way analysis of variance (ANOVA) and Student's t-test, after data transformation into the arcsine square root of the proportion, using the SigmaStat software (SPSS, Inc., Chicago, IL). C = HAM F-10 culture medium (negative control); FF = follicular fluid (1:1,000; positive control). Data are expressed as mean ± SE. a Significant differences versus negative control, $P<.05$.



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of Fig. 1B), therefore the chemotactic peaks observed at picomolar quantities of the solution of CBG + P resembles that observed when spermatozoa were exposed to P at the picomolar concentration range (Fig. 1A,B). Instead, at higher concentrations of CBG + P solution ($\geq$10 nM) most of P is tightly bound to its carrier protein (see estimated concentration in the upper part of Fig. 1B). Interestingly, the sperm chemotactic response toward 10 nM CBG + P solution (Fig. 1B) is elicited with lower concentrations of P than the much higher micromolar P needed to stimulate sperm chemotaxis (Fig. 1A), suggesting that CBG may modulate chemotaxis mediated by P. According to the free hormone hypothesis, the binding of hormone to CBG may serve as a tissue buffer, meaning that only unbound steroid is considered to be biologically active (12, 14). However, it is not the case for P action on spermatozoa, as it is well known that when P is bound to another carrier protein like bovine serum albumin (BSA), it can induce acrosome reaction (AR) (16), calcium mobilization (17), and chemotaxis (unpublished data) to the same extent than free P.

Human spermatozoa seems to express two P receptors on the cell surface (18), one with a high affinity site (K_d = ~600 pM), which appears to be specific for P binding, whereas the low affinity one (K_d = ~26 μM) also binds 11β-hydroxyandrostenedione (11-OHA) and 17α-hydroxyprogesterone (17-OHP) (18). Taking into account that [1] P is a physiological attractant that guides human spermatozoa mainly at picomolar concentrations, and at a lower extent at 1 μM (8), and [2] CBG, as well as P, is synthesized and secreted by cumulus oophorus cells (13), our results suggest that CBG would modulate the chemotactic activity of P at a nanomolar range, where both molecules are tightly bound. Because the low affinity P receptor can also bind other steroids at higher concentrations (micromolar), a hypothesis for the CBG chemotactic modulation of P could be based on a nonproper binding of P to its receptor due to a not suitable steric conformation of the binding site. If this is the case, the spatial arrangement of the CBG–P complex may help P have a better interaction with the low affinity P receptor, making it sensitive at lower concentration (CBG + P = 10 nM; bound P = 3 nM; Fig. 1B). Conversely, when spermatozoa are stimulated only with P, higher doses of the steroid (1 μM) are needed to stimulate the low affinity P receptor. Therefore, human acrosome reaction can be induced by micromolar P concentrations (19), but when the steroid is combined with CBG, a solution of CBG + P at a nanomolar concentration is enough to elicit the AR (20).

Considering that: [1] for the occurrence of chemotaxis, spermatozoa must sense a concentration gradient of the attractant, [2] along the cumulus oophorus there is a concentration gradient of P, and probably its carrier protein CBG, [3] human spermatozoa are chemotactically guided by P and a solution of CBG + P but not by CBG alone, and [4] P seems to be the sperm chemoattractant secreted by the cumulus cells, we propose a two-step model for sperm chemotactic guiding in the site of fertilization. First, at the surrounding of the cumulus oophorus, a gradient of low quantities of unbound P (at picomolar concentrations most P is unbound to CBG) may stimulate the high affinity P receptor, helping spermatozoa to find the egg–cumulus complex. Second, spermatozoa may sense a CBG–P complex gradient generated by a higher compound concentration, where CBG may modulate the binding of P to the low affinity P receptor, thus guiding spermatozoa through the cumulus mass to approximate them to the egg surface.

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